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PRELIMINARY OBSERVATIONS ON SUGAR CANE MYCORRHIZAE AND THEIR RELATIONSHIP TO ROOT DISEASES[1]

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INTRODUCTION

Up to the present time the subject of sugar cane mycorrhizae has been considerably overlooked or neglected, not only in relation to the complex and little known group of root diseases, but also as a problem which *per se* should be taken up.[2]

Only once in the literature of cane diseases is the subject mentioned, when Tryon (23) refers to a work of Treub (22), which unfortunately is unknown to the writer. Tryon, having found a *Pythium* in roots of canes attacked by the "top rot," states that Treub believed this fungus to be a root endophyte and, together with *Heterodera*, the cause of the "sereh." Constantin (8), also referring to Treub, states that the fungus of sugar cane mycorrhizae is known to be a *Pythium*, and in a later publication (9) gives his opinion to the effect that mycorrhizae might possibly be the cause of "sereh."

MORPHOLOGICAL OBSERVATIONS

An examination of longitudinal sections of the rootlets of sugar cane (not the transient rootlets of a cutting) shows that in about 80 per cent of the cases a root endophyte can be noticed in the root system, no matter what the health conditions of the plant may be. It appears that rootlets in their full activity do not show a mycorrhizal development. In the case of initial infection, in spite of the presence of said fungi, only mycelium develops. The development of the mycorrhizae increases as the rootlets become older, reaching a maximum at their maturity, that is, after the second and third month, rarely at the fourth. As a general rule, the mycorrhizal fungus continues to live, associated with other fungi, but as a saprophyte.

[1] The writer is indebted to Professor E. C. Stakman, Editor-in-Chief of PHYTOPATHOLOGY, for his suggestions and criticisms in the final preparation of the paper.

[2] These preliminary notes comprise the observation and experience accomplished during 18 months. The writer has in preparation a more detailed study on the subject, in which the results of experimental work together with full explanations of some of the topics treated in the present article will be discussed.

Likewise, the extra-radical development of the symbionts, very limited if any during the initial infection, is easily detected in old or decayed roots.

The two types of mycorrhizae, described by Peyronel (20) as "phycomycetous mycelium" and "rhizoctoneous mycelium," may be found as endophytes in sugar cane. It is not easy to differentiate between them if the infection is not in an advanced stage, and especially if the infected rootlets are abnormal either because of the absence of other vegetative organs of the fungi than the mycelium, or more often because of the presence of Hyphomycetes (especially Mucedineous and Tuberculariaceous), of Mucoraceae, and probably of the Hymenomycetoid mycelium which associate with the endophytic fungi in their extra-radical life.

The extra-radical mycelium of the phycomycetous endophyte is formed typically by hyphae of relatively uniform diameter which sometimes enlarge progressively, producing tufts of mycelium, chiefly in the bifurcations of the root. It is very common to find ramified or depressed hyphae, but they are seldom found in groups. When the fungus is cultured in a wet chamber, inducing the saprophytic development of the fungus, the formation of "vesicles" or "sporangia" may be detected, although they are rare in nature. These sporangia are more or less round or ovate and very similar to those previously described by several authors. Up to the present, the author has not been able to see them germinate.

The extra-radical behavior of the phycomycetous endophyte is entirely different from that of the rhizoctoneous endophyte. As a rule the fungus is imperceptible, except where it may be found adhering to the exterior of the rootlets.

The intra-radical development of the phycomycetous mycelium is also slow, but is easily followed in stained material. The mycelium enters the rootlets by penetrating the walls of the epidermal cells, never through the root hairs, and later, in cases of severe infection, spreads across the bark and through the parenchyma. The mycelium is sometimes intercellular, but in most cases intracellular, and generally spreads longitudinally with comparatively few ramifications. The hyphae are of quite uniform size, usually have a thick membrane, although it may sometimes be thin, and contain brilliant, thick protoplasm, as may be found in the extra-radical mycelium. The passage from one layer of cells to another seems to take place normally through the cross walls. Likewise the passage from the exterior to the interior of the roots is accomplished through these cross walls. Frequently it happens that the mycelium expands rapidly and completely fills a cell cavity, and even two or three adjoining cavities; in fact, it takes on a shape very similar to that shown in plates published by Peyronel (20). However, the writer has been unable to observe the repro-

duction of spores recently described by Peyronel (19), and it may be that the expansions are merely expansions of the mycelium.[3]

Infrequently the phycomycetous endophyte may exist alone in the rootlets, but only during the primary stage of the infection; generally the phycomycetous endophyte is associated with the rhizoctoneous endophyte, although the latter under certain conditions may constitute the only type of mycorrhizal fungus.

The type of fungus constituting the rhizoctoneous endophyte is not different from that studied by the several authors who have discussed the subject of mycorrhizae. Its extra-radical development in tender rootlets of healthy plants is as well pronounced as the intracellular. The hyphae are somewhat irregular, depressed, frequently anastomotic, hyaline to pale brown, and in their intra-radical life generally hyaline to yellow. The formation of Monilia-like chains is generally reduced, but is more abundant in wet soils; and the most favorable conditions for their development are found in a moist chamber at normal temperature. After the infection has started in the piliferous layer of the rootlet, stromata and all the intermediate stages in the development of hyphae are in evidence. The entrance of the endophyte into the rootlets is effected as in the case of the phycomycetous mycelium. The production of pectinase by a species of *Rhizoctonia* (*R. solani* Kühn) has already been demonstrated by Matsumoto (14).

In rootlets of healthy cane the Rhizoctonia mycelium does not present special characteristics worthy of mention. In diseased roots both mycelia (which may coexist even in the same cell) show comparatively greater development than in healthy roots, but of the two mycelia the Rhizoctonia is always more aggressive in spreading through the tissues. When the hyphae extend almost to the bundles, forming numerous intracellular enlargements, the root is severely affected. When the cortical portion presents longitudinal incisions or depressions it is often possible to disclose the union of mycelia, pseudo stromata, and sclerotia, especially in the central cylinder.

TAXONOMIC POSITION OF THE ENDOPHYTES

Although the writer has not yet been able to determine exactly the taxonomic position of all the endophytes of sugar cane, the subject may be discussed here briefly. Further work is being done.

That the endophyte of the rhizoctoneous type is a *Rhizoctonia* is clear from the appearance of the mycelium, both in the host and in culture. This agrees with the conclusions of several investigators, chiefly as a result of root diseases. Peyronel (20) isolated and cultivated *Rhizoctonia* from

[3] The irregular thickenings of the hyphae that sometimes are detected in the interior of cells present characteristics like those described by Bourne (3) in Porto Rico.

three Gramineae, and in these and other plants discovered forms somewhat similar to *Rhizoctonia repens* Bern. and to *R. lanuginosa* Bern. Previously, Janse (12) discovered in *Festuca ovina* an endophyte similar to that examined by Schlicht (21) in *Holcus lanatus*. Recently Jones (13) discovered mycorrhizae in *Panicum capillare*. Matz (15) observed *R. grisea* (Stevenson) Matz, *R. pallida* Matz, and *R. ferrugena* Matz in roots of sugar cane. Peltier (17) made inoculation experiments with a species of *Rhizoctonia* isolated by Edgerton in 1912.

In the present experiments the author was able to isolate two different species of *Rhizoctonia*, provisionally designated as strains A and B. Strain A is undoubtedly the most common in the canes under observation: out of 16 successful isolations, following the well-known practice of Burgeff (6) and Peyronel (20), from healthy canes of different varieties collected from different places, strain A appeared in 14 cases, strain B in 2. As already mentioned, however, the writer did not make a comparative study to ascertain the exact identity of the two strains.

The phycomycetous endophyte of sugar cane is possibly not a *Pythium*, although a *Pythium* has sometimes been isolated. On different occasions a *Pythium* has been reported as a parasite on the root of the sugar cane. The first observation was made by Treub (22) and the next by Wakker (24) in Java. In Porto Rico, Matz (15) isolated the previously mentioned *Rhizoctonias* and a *Pythium* which he believed to be *P. de baryanum*. Carpenter (7) in Hawaii believed a *Pythium* to be the agent causing the root disease (especially the "Lahaine disease") of cane and other plants. Bourne (3) studied a *Pythium* found in roots of sugar cane in Porto Rico, the *P. de baryanum* or *Nematosporangium aphanidermatum* (Ed.) Fitzpatr. (*Pythium butleri* Subramanan).

The literature on the endophytic characters of the *Pythium* is very limited. In addition to the observations of Treub (22) already mentioned, Bruchman (4) described an endophytic *Pythium* in the prothallium of *Lycopodium*.

In only 2 of our 16 tests with diseased roots of sugar cane were we able to isolate a *Pythium*. From its morphological character it might possibly be identified as *P. debaryanum*. Out of 18 tests with young roots of healthy cane this *Pythium* was discovered once when the phycomycetous endophyte was also present. On the other hand, in 3 of 10 experiments the writer was able to isolate the same fungus from roots so entirely invaded by various saprophytes that it was almost impossible to identify the phycomycetous endophyte with the aid of the microscope. Could the *Pythium* itself, which under different environmental conditions and at certain stages of growth of the rootlets is a parasite on sugar cane, be the phycomycetous

endophyte? It is impossible to answer this question without further study. From our experiments we are inclined to believe that an unknown phycomycetous endophyte and sometimes a parasitic *Pythium* may be discovered on roots of the sugar cane; the former can not be cultured artificially while the latter can be. This explains why the attempts to isolate the phycomycetous endophyte have resulted in the eventual discovery and culture of a *Pythium*, and that Treub, at a time when knowledge on the matter was not so advanced, could identify a parasitic *Pythium* with a phycomycetous endophyte.

Incidentally we might note that both *Panicum barbinode* and *P. maximum* have mycorrhizae, although the mycorrhization may not be constant and is often very limited; in the same plot of land may be found plants affected with endophytes, apparently of phycomycetous and rhizoctoneous types, and plants free from them. The presence of a *Rhizoctonia* different from strains A and B was demonstrated by culture experiments.

According to the investigations of Wakker (24), Tryon (23), and others, the rootlets of the diseased cane show a breaking-down of the cortical tissue, often during the second, third, or fourth months. The cortex falls off and the rootlets finally decay. Tryon, although not finding the situation abnormal, states that the entrance of saprophytes through the longitudinal ruptures of the bark fosters the decay of the rootlets.

A thorough study of this phenomenon shows that as a general rule the rhizoctoneous type of mycelium is present. The fungus can be grown as a saprophyte in a moist chamber. The presence of the mycelium can be detected if the rootlet is examined when it has become a pale brown color, before the falling off of the parenchyma. At a later stage the rootlet is invaded by other saprophytes which usually tend to mislead the observer. However, we are not in a position to state whether the functional inertia and the decay of the rootlets is caused by *Rhizoctonia*, as we have not been able to demonstrate it by actual experimental work. If the parasite is the rhizoctoneous mycelium, it is indeed a weak parasite, for, while the infection is initiated when the rootlets begin their activity (when the parasite is apparently harmless), the rootlets begin to show symptoms of weakness and decay after the second, third, or fourth month. If the rootlets are not apparently injured, they derive no advantage from the endophyte, and it is absolutely necessary to make experiments to define the case. Our experiments are not complete; therefore no conclusive evidence has been obtained.

Petri (18) and Peyronel (20) are of the opinion that the parasitism of the mycorrhizal fungi is a "refined" parasitism in which the fungus does not kill the host plant but stimulates the nutritive process. In an indirect way this may be possible in sugar cane, if we consider that the stimulus

would not be shown in the infected roots alone, but in the whole plant, which requires a superproduction of roots.[4] To find some reasonable explanation for this hypothesis, it would be necessary to do some experimental work with cane both with and without mycorrhizae, the experiments to continue until the canes mature. For the present, we conclude that the mycorrhizal fungi are apparently harmless to plants at an early age and harmful at a later stage, developing into a form of parasitism.

The tests with *Rhizoctonia*, especially with strain A, can easily be made under favorable conditions, as may be noted from the inoculation experiments made by Matz (15), Bourne (2), Peltier (17), *et al.* The difficulty will be to keep the soil aseptic without disturbing the transpiration of the plant which is correlated with the absorbing activity of the roots, principally when the cane reaches a height of 30 to 40 centimeters. The strain A of *Rhizoctonia* does well in sterilized soil rich in humus with a mixture of leaf mold and decayed stems. A great number of the rootlets are entirely invaded by the fungus as soon as they have attained full development and the appearance already mentioned. The fungus continues its development saprophytically in the soil, especially if it is kept wet at all times. The check plants in sterilized soil, without endophytic organism, grow just as well as the plants with the fungi during the limited duration of the experiments. As soon as conditions are rendered unfavorable for the development of the plants, chiefly through inefficient transpiration, the rootlets of plants grown in the presence of *Rhizoctonia* show an altered condition very similar to the normal retardation already described. The rootlet turns reddish and rapidly darkens at the tip; then the bark decays. Not only is this phenomenon constant and the cause of abnormal conditions in the plants, either owing to excess or lack of humidity; but it is also the first sign of disease in the cane, appearing when the above-ground portion of the cane does not yet indicate any abnormal state of the roots—even when the environmental conditions are unfavorable—although the most delicate plants may appear slightly weakened.

Experiments with plants grown under the same conditions of environment showed that the root system appeared, as a general rule, to be better developed in plants with endophytes than in plants free from mycorrhizae, either with more abundant ramifications or longer roots, but it is not possible to make a definite statement in this connection because the plants had not reached full maturity.

The test in which the phycomycetous endophyte was present was made in pots of sterilized soil in which pieces of roots had been allowed to rot

[4] A normal superproduction if we consider the endophitism and its effects as being a normal phenomenon.

and on which only the phycomycetous mycelium was known to exist. The mycelium of the endophyte could not be discovered in the roots of the canes planted. Cuttings previously disinfected externally were inoculated with a culture of a *Pythium*, under conditions which were not definitely controlled, but in which humidity played an important part. The fungus rapidly invaded the rootlets, even though they had not attained full development, and gradually spread through the cortical parenchyma. This fungus also produced, more or less uniformly, a diffused browning of the root and slow death, without generally producing decay; a phenomenon somewhat similar to mummification. Sometimes the rootlets, although infected, were not severely injured. The Pythium infection is probably less common than that of the *Rhizoctonia* strain A. The Pythium mycelium, chiefly at the time of initial infection, may easily be confused with the phycomycetous endophyte.

The plants artificially inoculated with the *Pythium* and those naturally infected with the phycomycetous endophyte may also be infected by the rhizoctoneous fungus by transplanting affected plants to pots of soil inoculated with *Rhizoctonia*. The infection of the roots inoculated with the *Pythium* takes place rapidly while it is slow in the case of the phycomycetous endophyte. The new mycelium develops profusely and after a few days the roots decay. However, in cuttings of cane inoculated with a culture of *Pythium* and grown in pots of soil inoculated with the *Rhizoctonia* strain A, the first fungus generally penetrates some rootlets, and on their complete development a severe infection of *Rhizoctonia* follows, either in the roots invaded by the *Pythium*, which then decay, or in healthy roots; and when the vitality of the plant declines, the endophyte apparently develops into a parasite. The intra-radical development of the *Rhizoctonia*, unlike that of *Pythium*, seems to take advantage of deficient humidity.

It is not easy to arrive at definite conclusions from our experiments; sometimes results are contradictory and incomplete. The rhizoctoneous mycelium in roots has the nature of an endophyte, and when the plant has been grown under poor conditions, or when the infection has had two or three months' duration, the fungus may turn into a parasite. The phycomycetous endophyte in rootlets does not develop fully and under ordinary conditions does not behave parasitically, although its presence fosters the development of the *Rhizoctonia* which is more virulent. The *Pythium* seems to be a true parasite which requires special environmental conditions for its development and action. In normal cases, and assuming that the three fungi exist in the soil, the *Pythium* would determine, under certain conditions, the outbreak of root diseases, probably being assisted in this by the contemporaneous development of the *Rhizoctonia*; while the latter, a

regular endophyte, might possibly be blamed for the general weakness of the roots, without causing a disease. The resistance of the cane to *Pythium* has not been clearly determined. In the case of *Rhizoctonia* the result was the formation of new roots, which is possibly an indirect stimulation caused by the same endophyte. The behavior of the phycomycetous endophyte, if this, as the writer believes, is different from the *Pythium* (in spite of some contradictory facts), is rather obscure. The fact that it is never found alone, but always in association with the *Rhizoctonia* or *Pythium* or with both, and the more luxuriant development and aggressive nature of the *Rhizoctonia,* account for the impossibility of distinguishing between the factors. Such being the case, only experiments under special control conditions may decide this question.

RELATIONS OF ENVIRONMENTAL CONDITIONS

Under normal conditions, the reaction of the cane against the infection of endophytes, as already explained, consists chiefly in the formation of new roots. An equilibrium is established between the physiological inactivity of the roots and the formation of new and active roots, that is, an equilibrium between the parasitic activity of the endophytes and the formative stimulus of the plant, and it is then that the influence of the environmental conditions need to be determined.[5]

As a direct result of what has been stated, any condition of the unfavorable environment which interferes with the equilibrium between the loss of activity of the roots, due to the parasitism of the endophytes and the formation of new roots, may be considered as the indirect cause of root disease.

It might be possible that favorable environmental conditions have little effect on the two kinds of fungi, assuming that even under unfavorable conditions they exist in the soil of sugar cane plantations, where organic matter is generally found, and that such conditions would tend to facilitate the saprophytic development of the fungi; but this possibility can not be accepted until the possibility of the virulence of the fungi is demonstrated or their special adaptation to the endophytic function under special conditions. Any condition which prevents the plant forming new roots and interferes with its metabolic activities favors the development of root disease. The plant dies when it is no longer able to compensate for the loss of affected rootlets. On the other hand, the conditions which favor the development and vegetative activity of the cane would likewise be unfavorable for the development of the root disease.

[5] In speaking of the parasitic activity of the endophytes, the writer has considered the joint action of the two types.

Effect of soil moisture

To a certain extent the ideas expressed above were verified by experimental work. Several cuttings were inoculated with *Rhizoctonia* strain A and the *Pythium* sp., and later planted in boxes, with the sole aim in mind of ascertaining the existence of the two fungi. In all the experiments a number of check plants were used. The plants were allowed to grow to a height of 20 centimeters under normal conditions; then a number of plants were deprived of water, some were watered abundantly every day, another lot of plants was transplanted to soil especially rich in organic matter (manure and leaf mold), a fourth set was transplanted to soil where the same fertilizers were applied moderately, and still another group was transplanted to soil where a moderate application of a mixture of phosphorus and nitrogen was administered. The check plants were growing without fertilizer, but were regularly watered.

Periodical observation of the roots under the microscope indicated that in normal environmental conditions the affected plants grew less than the check plants and the root system appeared slightly infected; about 50 per cent of the rootlets appeared infected by both fungi, with constant renewal of infection. Excessive water applied to the underground portion of the plants resulted in the death of 90 per cent during the period varying from 40 days to 3 months; practically all the rootlets were contaminated by both *Pythium* and *Rhizoctonia,* and the growth of new roots was less than in the check plants. All the unwatered plants died, showing a comparatively small percentage of rootlets invaded by the *Pythium* and the majority infected by the *Rhizoctonia,* the formation of new roots being next to impossible. The infection by *Rhizoctonia* alone, while not reducing to a minimum the vitality of the check plants, did not apparently injure the plants cultivated under excessive humidity during the first three months; after this period the plants showed a severe chlorosis, and in about four and a half months 50 per cent of them died. The endophyte ramified through a great portion of the rootlets; and the formation of new roots was at first regular and appeared to continue without difficulty. Under almost drought conditions the development of the plants was very limited and the death rate high (about 80 per cent); mycorrhization was heavy (not total), and the emission of new roots, particularly after the third month, very rare.

Effect of soil nutrients

The influence of fertilizers was not clearly shown. A probable increase of mycorrhizae was noted in plants grown in soil rich in organic matter, due to the rhizoctoneous endophyte, but there was apparently no change in the parasitism of the *Pythium.* The effect of fertilizing with mineral

matter was more definite: a vigorous general development of the cane, including the root system, was evident; there was also a comparatively smaller number of rootlets infected with the rhizoctoneous endophyte than in the check plants, and a very limited infection by the other fungus.

Effect of climatic and edaphic conditions

Several authors who have previously studied the root disease believed it to be due to, or favored by, different and contrary environmental conditions, as, for instance, excessive moisture or severe drought, or differences in physical or chemical composition of the soil, etc. These observations, apparently contradictory, may well be explained or accounted for by examining the relationship between plants and their environment and their relative quantitative values.

The excessive moisture of the soil, while not favorable to root activity nor interfering with the formation of new roots, favors the saprophytic development of fungi in the soil.[6] On the other hand, severe drought might constitute a favorable condition for root diseases, the development of the roots being very slow, as explained by Petri (18) in the study of the mycorrhizae of the olive tree. New roots are formed with some difficulty; consequently the plant weakens and can not offer high resistance. The fact that under such conditions the saprophytic life of the fungi is made difficult does not suffice to balance the slow and inefficient development of the root which appears to be the principal factor in resistance to rot.

The lack of soil cultivation which is noted in many sugar cane plantations contributes to the formation of a network of saprophytes, among which species of *Rhizoctonia* are very frequent. The lack of aeration in the soil tends to check the functional activities of the root. The organic matter in the soil affords a good substratum for the endophytic fungi, and organic matter together with green manure may facilitate the development of the root disease when the plants are not vigorous enough to counterbalance the action of the fungi.

The evil influence is especially felt when combined with other unfavorable conditions, such as a marked alkalinity or acidity in the soil. Alkaline soil constitutes the best medium for the saprophytic development of these fungi, apart from the fact that some varieties of cane do not thrive well in this type of soil. Although not having observed canes planted on acid soils, the writer is of the opinion that canes are predisposed toward root diseases

[6] The development of fungi, chiefly the Rhizoctonias, to a few centimeters depth in the vegetable layer of the soil is under such conditions extremely luxuriant. This fact was noted by several investigators, among whom is Peyronel (20); a similar observation has been made regarding the Phycomycetes by De Bruyn (5), Dufrénoy (10), *et al.*

because of the unfavorable influences of acid soils on the development of the root system.

Heavy soil seems to favor the development of roots if the amount of clay is not excessive. While a compact clay ground is unfavorable to root development, a soft clay loam is favorable to the plant, as well as a sandy loam that is rich in assimilable substances. The two types of soil frequently influence root development: on light soils roots grow deeper and sometimes the rootlets are apical; on heavy soils numerous lateral roots are formed which do not penetrate so deep into the soil. But we are unable to ascertain what relation exists between these two types of root development and the mycorrhization and root diseases.

As stated by Earle (11), it is possible that some varieties of cane under certain conditions show differences in susceptibility to the root disease, but there is no conclusive evidence. The writer has not carried out experiments with different varieties of cane grown under different environmental conditions.

PREVENTION OF ROOT DISEASES

The best and perhaps the only way to prevent root diseases, as previously explained, is to grow the plants under the conditions which are best adapted for their growth and vitality; consequently any undesirable agent should be eliminated and proper cultivation practiced whenever required.

Experiments were made to prevent infection by endophytic fungi and *Pythium* by means of a partial sterilization of the soil, using copper sulphate and sodium arsenate. Partial sterilization of the soil as a prevention against diseases and plant parasites was investigated by Miège (16) and by Bezzsonoff (1). Although these experiments are still being conducted, it is anticipated that little will be accomplished along this line. The *Pythium* is easily affected by the copper sulphate and also by the arsenic compound. The *Rhizoctonia* strain A shows little reaction to either compound with the dose applied. The effect of sterilization is of short duration and the benefits derived therefrom very limited. Sterilization might be of use before the planting operation but not during the growth of the plants. The direct control of the disease in the soil is impossible, and efforts should be directed toward prevention.

SUMMARY

1. These preliminary observations embody the results of experiments and investigations on the mycorrhizae of sugar cane and their relation to the root disease previously considered to be caused by Hymeniales.

2. Diseased or healthy rootlets of cane may be invaded by both a phycomycetous endophyte and a rhizoctoneous endophyte, or by the latter alone.

A *Pythium* sp. probably different from the phycomycetous endophyte may be found associated with it.

3. There are two strains of the rhizoctoneous endophyte, A and B.

4. The phycomycetous endophyte can not be cultured artificially, and therefore its systematic position can not be well defined; the rhizoctoneous endophyte was cultivated.

5. It may be possible that the "normal retardation and death of the rootlets of the sugar cane" in relation to the presence of the rhizoctoneous endophyte may at first be either neutral, or indirectly a stimulus to the formation of new rootlets, and later take on a parasitic nature. The behavior of the phycomycetous endophyte when alone is unknown. The *Pythium* appears to be a parasite at all times.

6. The death of rootlets previous to the normal retardation is undoubtedly related to the action of the endophytes, and the development of the root diseases results from the loss of equilibrium between the dying rootlets and new rootlets being formed.

7. Consequently any cause which hinders the rapid formation of new rootlets favors the outbreak of root diseases; excessive moisture, severe drought, high acidity or alkalinity in the soil, lack of cultivation, special physical or chemical composition of soils, etc., may be of decided importance.

8. Means of control against this type of root diseases do not show great possibilities. Growing the cane under the best conditions for development and vitality would tend to maintain the equilibrium between the functional inactivity of diseased rootlets and the formation of new ones.

9. Further research on the subject is being done by the writer, and the results of succeeding investigations will be published at a later date.

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PREDISPOSITION OF SUGAR-BEETS TO LATE ROOTROT[1]

GEORGE STEWART[2] AND D. W. PITTMAN[3]

During the last few years sugar-beets in the Rocky Mountain region have been subjected to a series of disastrous epidemics. Of these, much the most important, if not the only ones of serious consequence, are curly-top and late rootrot.

The sugar-beet leafhopper (*Euttetix tenellus*), or white fly, as it is locally designated, probably transmits a virus. Because of the characteristic curled and dwarfed leaf the disease is known as curly-top. On account of the generally unsuccessful attempts either to control or to prevent curly-top, and on account of reported observation of some resistance in certain strains,[4] there is a general feeling that plant-breeding is the one really promising method of attack yet to be tried for that trouble. The genetic search for a strain of sugar-beet resistant to the virus transmitted by the leafhopper is likely to occupy a considerable part of the future research program for sugar-beets.

Many field men of sugar companies say that proper maintenance of soil productivity and of optimum moisture conditions for rapid growth influence, in a marked degree, the injury due to curly-top. Careful observation by various workers of the Utah Agricultural Experiment Station, as well as by other scientific workers, show that this position is probably untenable. In the last ten years there have been five epidemics in the Utah-Idaho region —in 1917, 1919, 1921, 1924, and 1926. In addition, there was much loss in 1923. The trouble is rather common throughout the Rocky Mountain region. A similar and perhaps identical condition has caused much trouble in Michigan.

In 1926 curly-top was especially abundant. To it was popularly attributed all of the injury, not only that actually caused by curly-top, but also that caused by late rootrot, which in many parts of Utah and southeastern Idaho probably accounted for considerably more than half the trouble.

[1] Contribution from the Department of Agronomy, Utah Agricultural Experiment Station. Approved for publication by Director, November 12, 1927.

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[4] Carsner, E. Resistance of sugar-beets to curly-top. U. S. Dept. Agr. Dept. Circ. 383. 1926.

Since many farmers, and even many fieldmen of the sugar corporations, have failed to distinguish between late rootrot and curly-top which occurred simultaneously in the same field, it is not surprising that they interpreted the effect of good soil treatments on rootrot as an effect on curly-top. In 1926 there was only about 1 per cent loss due to curly-top on all of the sugar-beet plats of the Central Experimental Farm, North Logan. The fact that production in some plats was extremely high and in others extremely low affected the percentage of infection not at all.

In the case of rootrot, however, the predisposing effects of low soil productivity and of unfavorable soil moisture are clear-cut.

This paper has to do chiefly with the disease herein called late rootrot for the want of a better name. Pathologists who studied the epidemic in 1921 reported that the rot begins in June or July at the tips of the rootlets, which, as the rot progresses, begin to turn brown and become dry much as if scorched by a fire, the outer leaves being first affected (Fig. 1). The tops then die and become entirely prostrate. The tap root of the affected beet rots rapidly, usually a brownish-gray, rather dry mass in some cases and in

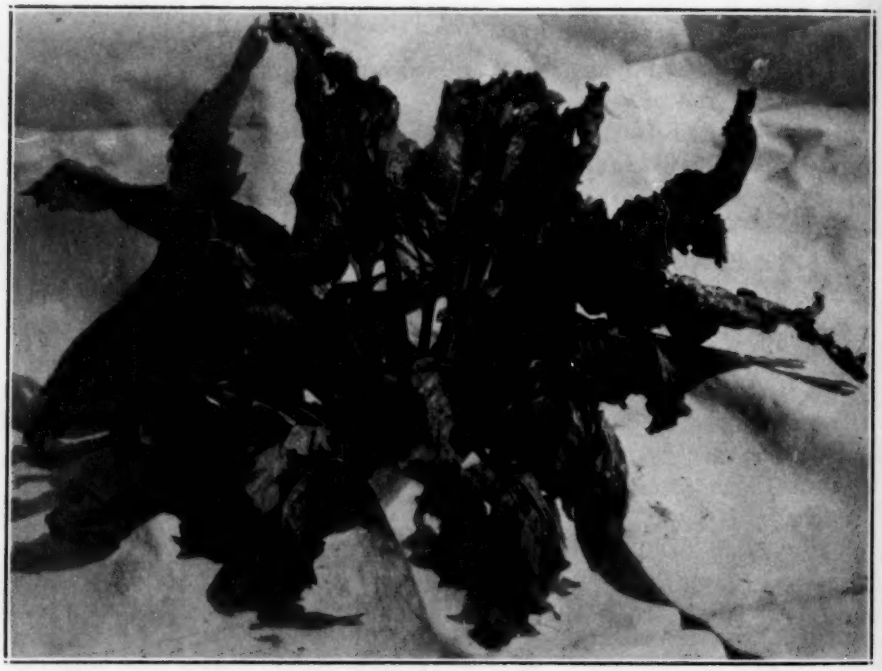

FIG. 1. General appearance of a sugar-beet plant in the early stages of late rootrot. The top takes on a burned appearance which spreads rapidly in epidemic years.

others a soft, dark-colored, mushy mass. During the later stages *Phoma betae* Frank and various Fusaria have been isolated,[5] sometimes singly and sometimes conjointly. Other organisms occur also. It was thought likely by pathologists that the initial trouble came earlier in the season and was probably due to some other cause, the *Phoma* and the *Fusarium* coming in as secondary invaders. Whatever the original cause may be, the decrease in vigor of the plant resultant on unfavorable moisture and fertility conditions, is closely associated.

In the epidemic year of 1924 the fields were rather generally damaged. In 1925 all beets on land that was cared for even reasonably well continued in a fresh, vigorous condition up to the harvest. In 1926, following a winter of little rainfall, serious trouble was clearly predicable in April, after a survey of the snow on the watersheds had been made. Injury began almost immediately. Seedbeds were dry; and where irrigation water was not promptly applied, and in many cases where it was, the leaves appeared unthrifty. More than four-fifths of the fields were affected: the leaves began to turn brown in early June; in two weeks, plants were dying in spots which increased rapidly in size. By August, many fields were nearly 100 per cent brown and dying, the trouble being greatly accentuated by a season of drouth in which the irrigation water available was only about 50 per cent of the normal amount.

With fully 80 per cent of the fields attacked, and the damage ranging from a trace to 100 per cent, the total loss mounted rapidly. The average yield for Cache Valley, Utah, in 1925, was 16¼ tons an acre; in 1924, only 7 tons; and in 1926 only 9 tons. The normal is about 12 tons. Thus the year 1925 was very favorable and 1924 and 1926 extremely unfavorable. The returns from sugar-beets in Cache Valley for 1926 alone were about a half million dollars below normal. In Utah and Idaho, it could not have been far from 5 or 6 millions below par. The 1924 loss was even greater.[6]

Part of the loss was due to curly-top, principally so in the southern extension of the sugar-beet area of Utah and in the western extension in Idaho. In Cache Valley, Utah, however, it was predominantly due to late rootrot.

A field survey in 1921 by the Plant Pathology Department, and also one in 1921 and 1923 by the Agronomy Department, of the Utah Agricultural Experiment Station, showed the trouble to be related to soil moisture and to soil productivity. Results of the Plant Pathology Department survey

[5] Thanks are due to Dr. B. L. Richards, Plant Pathologist of the Utah Agricultural Experiment Station, and to Dr. C. M. Tompkins, of the Office of Sugar Plant Investigations, U. S. D. A., for identifications of rot organisms in late-season stages.

[6] Thanks for these figures are due to the agriculturists of the Cache Valley unit of the Amalgamated Sugar Company, who kindly made them available.

TABLE 1.—*Soil treatments and acre-yields of sugar-beets on plats of low, intermediate, and high productivity in the two years of serious late rootrot injury—1923 and 1926*

Plat numbers	Soil treatment	Acre-yield in tons	
		1923	1926
	Low productivity plats		
5G, 6G	Four-year rotation, clover, potatoes, beets, wheat (no manure)	6.5	0.7
13G, 17G	Alternate wheat–beets, no manure	2.6	0.9
16G, 20G	Alternate wheat–beets, ½ green manure[a]	3.1	1.7
35G	Continuous beets, no manure (check)	8.5	4.1
34G, 38G	Alternate peas–beets, no manure	9.6	4.7
39G	Continuous beets, no manure (check)	9.0	5.2
36G, 40G	Alternate peas–beets, green manure	6.7	7.4
46G	Continuous beets, no manure (check)	7.1	9.1
47G	Alternate corn–beets, no manure		2.6
51G, 54G	Six-year rotation wheat–potatoes, 2 yrs. peas, 2 yrs. beets (1st yr.) no manure	2.4	1.6
52G, 55G	do (2d yr.) do	3.1	2.6
9G, 10G	Four-year rotation, clover, potatoes, beets, wheat, ¼ manure[a]	6.4	7.6
	Average	5.1	3.6
	Intermediate productivity plats		
14G, 18G	Alternate wheat–beets, ½ manure[a]	10.5	15.0
15G, 19G	Alternate wheat–beets, ¼ manure[a]	10.0	11.5
86G, 83G	Ten-year rotation, alfalfa, potatoes, beets, peas, wheat, 3/10 manure[a]	16.0	13.0
88G, 85G	do do	9.6	18.8
96G, 93G	do do	19.6	15.8
98G, 95G	do do	15.7	25.7
	Average	10.9	16.6

TABLE 1.—*Continued*

Plat numbers	Soil treatment	Acre-yield in tons	
		1923	1926
	High productivity plats		
22G, 25G	Seven-year rotation, alfalfa, oats, beets, 2/7 manure[a]	21.8	20.2
23G, 26G	do (1st year) 2/7 manure[a]	13.6	18.4
28G	Continuous beets—40 tons manure annually	27.3	29.0
29G	do —15 do	23.7	25.4
30G	do — 5 do	20.6	23.2
44G	do —30 do	26.1	28.3
45G	do —10 do	22.2	21.3
48G	do —20 do	11.1	23.6
37G, 49G	Alternate peas–beets, ½ manure[a]	12.7	20.0
50G	Continuous beets, (20 tons manure, alternate years)		
46F, 43F	Ten-year rotation, alfalfa, beets, 3/10 manure[a]	19.0	21.9
48F, 45F	do do	24.9	25.9
56F, 53F	do do	21.4	24.4
58F, 55F	do do	24.8	23.1
66F, 63F	do do	15.0	24.4
68F, 65F	do do	21.3	20.4
76F, 73F	do do	19.8	18.6
78F, 75F	do do	20.8	24.6
	Average	21.6	24.6

[a] The lower figure of the fraction occurring in front of the word "manure" indicates the length of the crop rotation used on the plat series, and the upper figure of the fraction the number of years in the rotation in which manure was applied at the rate of 10 tons to the acre. Thus, "2/7 manure" means that in a 7-year rotation manure was applied twice, usually on sugar-beets .

were published locally in 1922;[7] those of the Agronomy Department survey form part of this article, having been withheld until more experimental plat data became available. These were obtained in 1923 and 1926. The data of 1924 were not sufficiently detailed to permit a close study.

EFFECT OF SOIL PRODUCTIVITY

For a number of years sugar-beets have been grown under various rotation and manuring treatments at the Central Experimental Farm, North Logan, of the Utah Experiment Station. In table 1 are given the yields during two epidemic years, 1923 and 1926. These are grouped into three classes according to the productivity of the soil in non-epidemic years, as follows: (*1*) low productivity, (*2*) intermediate productivity, (*3*) high productivity. Many of the treatments began as late as 1922; therefore the soils had not reached their true state of productivity by 1923. Some of these treatments, which began in 1922 on depleted land, after sufficient time will bring the soil into a condition of high productivity.

Since, however, there has not yet been sufficient time to accomplish this, the plats receiving such treatments are ranked as of low or of medium productivity. These plats in the lower section of the table which have "F" as part of their number designation, even though treatments began only in 1922, are classified as being in a condition of high productivity because the land had been manured for 20 years previously with about 10 tons an acre of farm manure annually.

The continuous beet plats that were manured received an application every year. As the effort here is to study the effect of productivity on the prevalence of rootrot disease rather than to discover the particular treatments that bring about conditions of varying productivity, this latter question is not here considered.

Table 2 shows for 1926 the number of beets harvested, the number of diseased beets about three weeks before harvest, and the percentage of beets diseased with rootrot when the number harvested on each plat is regarded as 100 per cent.

On the plats of low productivity the percentage of beets affected varied from more than 71 to less than 9 per cent. The mean was 29 per cent. For the plats of intermediate productivity, the range was from 6 per cent to just over 2 per cent, the mean being 3.63 per cent. On the plats of high productivity, the range was from about 4.9 per cent to about 0.25 per cent, with the mean at 1.35 per cent.

The wide gaps between 1.4 per cent infection, 3.6 per cent infection, and 29 per cent infection are so striking as to leave no doubt of real differences.

[7] Richards, B. L. Beet disease situation (in Utah) during 1921. Utah Farmer **18**: May 20, May 27, 1922.

TABLE 2.—*Number of beets, number of diseased beets, and the percentage of beets infected with rootrot disease on plats of low, intermediate, and high soil productivity. Counts taken Sept. 15, 1926*

Low productivity plats				Intermediate productivity plats				High productivity plats			
Plat no.	Total no. beets	No. beets diseased	Per cent rootrot	Plat no.	Total no. beets	No. beets diseased	Per cent rootrot	Plat no.	Total no. beets	No. beets diseased	Per cent rootrot
6G	406	291	71.67	18G	657	23	3.50	25G	838	41	4.89
10G	733	91	12.41	19G	711	27	3.80	26G	748	12	1.61
17G	335	208	62.09	49G	728	20	2.75	28G	769	2	0.26
20G	460	132	28.70	83G	796	49	6.16	29G	833	2	0.24
35G	547	122	22.30	85G	810	26	3.21	30G	676	26	3.85
38G	435	87	16.51	93G	821	30	3.65	44G	861	3	0.35
39G	510	96	18.82	95G	814	18	2.21	45G	668[a]	23	3.44[a]
40G	734	130	17.71					48G	840	10	1.19
46G	605	51	8.43					50G	875	13	1.49
47G	479	169	35.73					43F	790	7	0.89
54G	630	347	55.08					45F	835	7	0.84
55G	665	146	25.84					53F	885	11	1.24
								55F	710	5	0.70
								63F	864	9	1.04
								65F	790	6	0.76
								73F	857	5	0.58
								75F	927	4	0.43
Ave.	545	155.83	28.6		762	27.57	3.63		809	10.94	1.35

[a] Infected with nematodes.

Even these wide differences, however, are only part of the story. The other part lies in the actual number of beets harvested. The plats were the same size and should have produced approximately equal numbers of roots. The mean expectation of beets on the well-manured plats is about 800 to the 1/25-acre. The mean of 809 beets on the plats of high productivity closely approaches this number. The mean of 762 beets on the plats of intermediate productivity shows a loss of 47 beets, or 6 per cent, as compared with the number on the highly productive plats. On the plats of low productivity there was a mean of only 545 beets, or a decrease of 264 beets, which is a loss of 33 per cent. These losses represent the number of beets which died early enough in the season not to be counted on September 15 when the official disease count was made. In other words, 33 per cent of the beets died and rotted early enough to have disappeared before September 15. In addition, 29 per cent of the remaining 67 per cent were observed to be diseased on that date. Computation shows this loss due to rootrot to be a total of about 52 per cent of the number of beets that should have been harvested. On the plats of intermediate productivity the total loss was about 9.5 per cent. The extent of loss due to rootrot was therefore about 1.5, 9.5, and 52 per cent on the plats of low, intermediate, and high productivity, respectively.

The epidemic of 1923 began early in the season, and the infected areas were more widespread. At harvest practically all roots were diseased on land low in productivity or poor in soil moisture, that is, either where soils were too dry or too wet. These conditions were studied on the Central Experimental Farm (North Logan) for soil productivity in the manner already described for 1926 as well as for soil moisture.

The total number of beets harvested about October 20, the number of diseased beets counted on October 8, and the percentage of diseased beets are shown in table 3. On the plats of low productivity practically all the plants were diseased, and many were dead and rotting on October 8. There were therefore more beets in many of the plats than were in a condition to be harvested two weeks later when the actual harvest was made. This is shown by more than 100 per cent being classified as diseased on some plats.

In the plats of low soil productivity there were only three of eleven plats where the percentage of diseased beets was not 100 or more. On the basis of total beets at harvest, the average was 102 per cent, which means that many beets rotted completely. In the plats of intermediate productivity the percentage diseased was 69, whereas it was only 5.3 in the plats of high productivity. In addition, there was a decrease in total number of beets of 16 and of 32 per cent for the plats of intermediate and low productivity, respectively. This means an infection of almost 100 per cent, of about 75 per

TABLE 3.—*Total number of beets, number of diseased beets, and percentage of beets injured by rootrot disease on plats of low, intermediate, and high soil productivity. Counts taken on October 8, 1923. Crop harvested October 20, 1923*

Low productivity plats				Intermediate productivity plats				High productivity plats			
Plat no.	No. plants diseased	Total no. beets at harvest	Per cent root-rot[a]	Plat no.	No. dead and diseased beets	Total no. beets at harvest	Per cent root-rot[a]	Plat no.	No. dead and diseased beets	Total no. beets at harvest	Per cent root-rot[a]
5G	904	844	107	14G	822	787	104	22G	27	958	3
9G	846	740	114	15G	842	836	101	23G	49	504	10
13G	765	564	136	37G	759	808	94	28G	6	875	1
16G	794	398	199	86G	74	944	8	29G	16	917	2
34G	847	846	100	88G	140	578	24	30G	40	838	5
35G	850	808	105	96G	.....	745	....	44G	18	1092	2
36G	655	649	101	98G	.....	666	....	45G	36	944	4
39G	192	688	28					48G	155	809	19
46G	140	581	24					50G	85	884	10
51G	518	328	158					46F	....	954	....
52G	392	335	117					48F	....	860	....
								56F	....	967	....
								58F	....	909	....
								66F	....	1022	....
								68F	....	985	....
								76F	....	923	....
								78F	....	974	....
Ave.	627	616	102		527	766	68.8		48	907	5.3

[a] On basis of total number of beets at harvest.

cent, and of about 5 per cent respectively, on the plats of low, of medium, and of high soil productivity.

The differences in susceptibility between the beets on the three grades of plats are really greater than the figures indicate. Plats 14G and 15G really belong in the low-productivity group for the year 1923, as their treatments began only in 1922, previous to which the land was badly depleted by 11 years of continuous small-grain growing without manure. Similarly, plat 48G should be ranked in the intermediate group instead of the high group on account of its having been manured only twice—in 1922 and in 1923—after 11 years of depletion by small grains.

The current season (1927) has been rather favorable to sugar-beets and not an epidemic year for rootrot. However, there has occurred a light attack of rootrot. This is fortunate, as it has been possible to discern slight attacks, which in epidemic years, or in years generally unfavorable to beets, would probably not have been observed. At the time of writing, harvest was some time off and the total number of beets harvested was not available. The percentage attacked by rootrot is calculated on the total number of beets in the plats on October 4, when the count was made. As extremely few beets were dying, this number is accurate within a minute fraction of 1 per cent. The data for 1927 are given in table 4.

The degree of injury on individual beets on the plats of low productivity was much greater than on the other two groups. The differences between 42.8, 6.7, and 2.3 per cent for the plats of low, intermediate, and high productivity again conform to the effects in the epidemic years.

TABLE 4.—*Percentage of beets attacked by rootrot in plats of low, intermediate, and high soil productivity. Counts taken October 4, 1927. Percentage on basis of total number of beets just before harvest*

Low productivity plats		Intermediate productivity plats		High productivity plats	
Plat no.	Per cent diseased	Plat no.	Per cent diseased	Plat no.	Per cent diseased
7G	46.2	14G	9.6	26G	2.2
9G	13.5	15G	9.9	27G	4.9
13G	60.5	82G	5.4	28G	0.0
16G	26.7	84G	9.7	29G	0.6
34G	25.7	92G	2.9	30G	0.4
35G	31.2	94G	2.4	37G	5.9
36G	55.8			48G	3.1
39G	42.6			50G	1.5
53G	79.6				
54G	46.1				
Average	42.8		6.7		2.3

EFFECT OF SOIL MOISTURE

During the season of 1923 the rootrot developed early, that is, before and shortly after thinning time. Careful observations were made in the beet fields of the following districts: Logan, North Logan, Hyde Park, Smithfield, Lewiston, Benson, and College. The order given is that recorded on a trip circuit from the Experiment Station and return.

The disease showed in spots in some fields and as blanket infection in others. The field studies were limited to those fields in which there were spots of diseased beets and spots of healthy beets.

The data given in the fifth column of table 5 were obtained by averaging five counts in each field where the moisture conditions were unfavorable for the beets. The data in the sixth column represent averages of five counts in

TABLE 5.—*The effect of soil moisture on the development of rootrot of sugar-beets during the seedling stages. Observations made in July, 1923, in Cache Valley, Utah*

Locality	No. of acres	Occurrence of rootrot	Low soil moisture		Per cent rootrot where soil moisture was high
			Cause	Per cent rootrot	
1. Logan	3.5	Entire field	Spring-plowed alfalfa	87	0
2. Logan	8.0	Lower end only	Not well irrigated	54	18
3. Logan	4.5	Spots	Gravelly soil	61	9
4. Logan	4.0	Spots	Gravelly soil	49	13
5. Logan	1.5	One side	Coarse manure	73	6
6. North Logan	12.6	Large spot	Gravel subsoil	59	8
7. North Logan	11.0	Middle	Steep—water runs rapidly	70	3
8. North Logan	7.0	Top half	Heavy grain stubble	46	7
9. North Logan	2.5	Entire field	Delayed irrigation	55	0
10. North Logan	15.0	Bottom only	Not well irrigated	65	14
11. North Logan	5.0	Bottom only	Flooded at bottom	34	11
12. Hyde Park	20.0	Entire field	Poor packing	39	0
13. Hyde Park	4.0	One side	Cloddy soil	67	5
14. Hyde Park	13.5	Spots	No cause visible	78	21
15. Smithfield	3.0	Spots	Gravelly soil	39	12
16. Smithfield	3.0	Spots	Gravelly soil	61	7
17. Smithfield	7.0	Upper half	Steep slope	66	14
18. Smithfield	18.0	One side	Alfalfa sod	59	8
19. Lewiston	60.0	Large spots	Some high, some low and wet	85	20
20. Lewiston	20.0	Lower end	Wet land	90	10
21. Lewiston	22.0	Entire area	Recent plowing	63	9
22. Lewiston	9.0	Spots	Long grain stubble	44	14
23. Benson	10.0	9/10 of field	Wet land	99	15
24. Benson	11.5	Bottom half	Very compact—some alkali	75	15
25. College	30.0	3/4 of field	Wet land	50	4
26. College	14.5	1/2 of field	Alfalfa sod	35	2
27. College	5.5	Spots	Excessively compact	45	8
			Ave.	61.0	9.4

parts of the same fields where the moisture conditions were favorable for beets. The figures for each particular field therefore are directly comparable.

The data reported in table 5 indicate that six times as many beets were diseased where the moisture conditions were unfavorable for beets as where satisfactory moisture conditions prevailed.

More general observations were made in several other counties of the state. Actual counts were not taken, but careful general observations were made in several fields of each county and the percentage of rootrot estimated. These data correspond roughly with those obtained in Cache County by carefully replicated counts. Table 6 summarizes the estimated

TABLE 6.—*The effect of soil moisture on the development of rootrot of sugar-beets in several counties of Utah and one county of Idaho. (No field counts taken—farmers not consulted)*

County	No. fields	Per cent soil with unfavorable moisture (based on total area)	Per cent rootrot when soil moisture was unfavorable	Per cent rootrot when soil moisture was favorable
Boxelder	9	25	75	10
Weber	4	50	50	10
Davis	7	35	50	15
Salt Lake	4	40	60	20
Utah	18	20	50	15
Sanpete	3	5	20	2
Sevier	7	2	15	Trace
Franklin (Idaho)	7	30	35	5
		Ave.	44.4	9.6

percentages of rootrot. There seems to have been almost five times as much disease in the spots of unfavorable moisture as in favorable spots.

There seems, therefore, to be good reason for concluding that unfavorable soil moisture conditions, particularly early in the season, predispose sugar-beets to attacks by rootrot.

Another set of data from the experimental plats also bears on this question of predisposition of sugar-beets to rootrot. Table 7 shows the effect of

TABLE 7.—*The effect of manure on development of rootrot in sugar-beets following other crops and following beets. Logan, Utah, 1923*

Crop rotation	Per cent rootrot	
	Manure	No manure
First year beets after other crop	94	8.0
Second year beets after other crop	51	3.0
Continuous beet crops	46	2.5

manure which is modified in a smaller way by the crop-rotation treatments.

There are great differences between manured and unmanured beets (Figs. 2 and 3). There is regularly about 12 to 15 times as much disease in beets on unmanured land. There was only about one-half as much disease when the beets followed beets as when beets followed other crops—in this case alfalfa, peas, and small grain. It is thought that the partially decomposed stubble residues from crops other than beets prevented optimum moisture or nitrate conditions from being established in the soil.

FIG. 2. Foreground: well manured sugar-beets in epidemic year. Back: poorly fertilized beets. All other conditions except soil treatment were equal.

SUMMARY AND CONCLUSIONS

1. Plats on the Central Experimental Farm of the Utah Experiment Station were grouped into classes of high, intermediate, and low productivity according to their yield of sugar-beets in non-epidemic years.

2. There were no differences in the degree of injury by curly-top on plats varying in soil productivity. Not nearly so many data were obtained regarding curly-top as regarding late rootrot.

3. There were, however, vast differences in the degree of injury by rootrot. These differences were permanent and outstanding. All plats low in soil productivity were heavily injured in both years when rootrot was bad. Plats of intermediate soil productivity were injured to an intermediate

FIG. 3. Yield of sugar-beets from 1/25-acre plats in 1921 at Central Experimental Farm, Logan, Utah. Left: actual beets from one entire plat heavily manured. Right: actual beets (on box) from one entire plat not manured at all. All other treatments were the same.

degree, and plats of high productivity were practically uninjured even when immediately adjacent, on every side, to plats fully infected.

4. Low productivity predisposes sugar-beets to the attack of rootrot so definitely and so strongly as to make good soil treatment, especially the use of farm manure and of reasonable crop rotation, the only control method the farmer needs to consider.

5. Unfavorable (either too dry or too wet) soil moisture conditions, especially early in the season, predisposed the beets to rootrot definitely, strongly, and almost without exception.

6. The maintenance of proper soil moisture conditions largely, if not entirely, overcomes this predisposition. In addition, soil moisture is more readily maintained in soils properly manured and rotated.

UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

THE PRODUCTION OF AGGLUTININS BY PHYTOPATHOGENIC BACTERIA[1]

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Researches in immunological and serological fields have been principally confined to animal life. The impetus to these researches has been the necessity of preserving human life and finding out the exact relations of antigens and antibodies to their hosts. The use of these serological methods in relation to plant diseases has been almost entirely neglected. Until recently workers have confined themselves to animal parasites in their relation to immune body formation.

Zipfel (7), as late as 1912, was probably the first to produce anti-sera by using phytopathogenic bacterial antigens. He was concerned with the relations of the various legume nodule bacteria, isolated from various hosts, to each other. Klimmer and Kreuger (4), working also with legume bacilli, found that from different species affected with the type of organism commonly known as *Bacillus radicicola,* closely related organisms were obtained, which proved to be specific for the host from which they were isolated when treated with antisera of the blood of rabbits. That is, each host had a specific bacterium which closely resembled the type, *B. radicicola.*

Jensen (3) produced antisera against forms of *Bacterium tumefaciens* S. and T., the widespread crown gall-causing organism. Paine and Lacey (5), by the use of antisera, showed the relationship of various organisms isolated from a diseased bean leaf. St. John-Brookes, Nain, and Rhodes (6) recently published a very excellent paper on the relations of many plant parasites to each other. Thirty-nine parasites were used. Antisera were produced against each, and cross agglutination was observed. These parasites came from various sources and fell into three groups, the yellow, white, and fluorescent forms. The investigators noticed considerable cross-agglutination among the members of each group, but not between the groups. Work of this nature will go a long way in clarifying the field of bacteriology as related to plant diseases. Very little is known as to the relations of the various similar plant disease organisms. There are many plant disease organisms that appear to be one and the same, but have been described as different species because they cause a disease on a different host.

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The above workers were all concerned with the relation of the specificity of isolated organisms. That organisms react so specifically with their antisera makes identification of these species absolute when the antisera are used in proper dilutions.

The writer's researches concern the bacterial organisms causing the gummosis of stone fruit trees. More than one organism appears to be active in producing this disease, although the literature on the subject describes only one species. Griffin (2) has described the organism *Pseudomonas cerasus* as causing this disease. The writer has discovered that at least two organisms, so closely related to each other that often it is impossible to tell them apart, are concerned in the disease formation. The writer designates these as *Ps. cerasus* var. 28 and 29. The only apparent difference is the production of a fluorescent pigment in liquid and solid media by one of them. This pigment is not always produced, and it is often difficult to separate the two organisms. Both cause the typical bacterial gummosis lesions when inoculated into healthy trees. As neither of these parasites could be easily distinguished, it became necessary to look for something more specific. If an agglutinating anti-serum could be produced against these parasites, then this method offered the means of differentiating and at the same time absolutely determining whether these were two species or whether they were the same organism. At the same time these antisera could be used in the study of the epidemiological relations. The persistence of the organisms in the soil—artificially and naturally, their persistence in old cankers, occurrence during the whole of the year in diseased trees, the part that certain orchard insects might have in the dissemination of these organisms and many other relations could be studied more readily with such sera, as all of the above studies would necessitate the collection of many bacterial cultures. The desired data could then be derived from the identification by use of the potent antisera.

The writer was also concerned with *Bacterium maculicolum* which causes the spot disease of cauliflower heads. This organism and the two varieties of *Ps. cerasus* causing the bacterial gummosis of stone fruit trees were used in the production of agglutinating antisera.

EXPERIMENTAL DATA

The production of an agglutinating antiserum is in itself normally not a difficult procedure. The methods of producing agglutinating antisera are numerous. The method used in these experiments follows that described by Gay and Fitzgerald (1).

Experiment I

Methods. The organisms were grown on Bacto-peptone-beef bouillon-agar slants, having a reaction of pH 6.8. The cultures were incubated at

room temperature. At the end of 48 hours, the resultant growth was suspended in 10 cc. of physiological salt solution (0.85 per cent). The suspension was then treated with enough 5.0 per cent phenol solution to bring the mixture to 0.25 per cent phenol. The phenolized cultures were allowed to stand for several hours before using, to allow the organisms to react with the phenol.

The injections were given intravenously in the amounts indicated in the animal charts. Preliminary bleeding to determine the course of the reaction was made from the marginal vein of the ear.

Rabbits were used for the experiment. Fairly large rabbits, weighing approximately 2,500 grams, gave the best results. One rabbit was used for each antigen. This experiment was a preliminary one, and the use of many rabbits seemed out of the question.

Results. The results were disappointing. In no case did the rabbits give potent antisera against the antigen. The experiment was carried on for almost a month, but during this time the titre did not reach a high point.

The cauliflower organism, *Bacterium maculicolum* McCulloch, apparently gives off a toxic substance. The first rabbit used against this antigen succumbed after five days. The organism used was dead, for on plates poured from the suspension there was no growth for 10 days. As a result of the death of this rabbit only the two gummosis organisms could be tested in the experiment. Charts 1, 2, and 3 show the course of the reactions.

Animal chart 1.

Rabbit 846.

Antigen, *B. maculicolum* (cauliflower organism) phenolized.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Jan. 26	*B. maculicolum*	0.5 cc. (i. v.)	Excellent	2800
Jan. 27	do	1.0 cc. (i. v.)	Good	2750
Jan. 28	do	1.0 cc. (i. v.)	Poor	2600
Feb. 1	.....	.....	Dead	.....

Post mortem was made too long after death. The animal, before it died, had an emaciated appearance, clots of blood could be seen in the veins of the ear, and at the site of inoculation a large black necrotic area had formed. Stools that formed were hard.

Animal chart 2.
Rabbit 847.
Antigen, *Pseudomonas cerasus* 28; phenolized.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Jan. 26	28	0.5 cc. (i. v.)	Excellent	2200
Jan. 27	28	1.0 cc. (i. v.)	do	2100
Jan. 28	28	do	do	2100
Feb. 2	28	do	do	2200
Feb. 3	28	do	do	2250
Feb. 4	28	do	do	2200
Feb. 10	Bled from the heart	Titre, 1–160	do	2380
Feb. 11	28	1.0 cc. (i. v.)	do	2350
Feb. 16	28	1.5 cc. (i. v.)	do	2400
Feb. 17	28	do	do	2380
Feb. 18	28	do	do	2380
Feb. 24	Bled from ear	Titre, 1–160	do	2500
Feb. 25	Bled from heart	Titre, 1–320	do	2500

Normal serum obtained on the first day did not give a titre with the antigen.

Animal chart 3.
Rabbit 848.
Antigen, *Pseudomonas cerasus* 29; phenolized.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Jan. 26	29	0.5 cc. (i. v.)	Excellent	2100
Jan. 27	29	1.0 cc. (i. v.)	do	2000
Jan. 28	29	do	do	2120
Feb. 2	29	do	do	2130
Feb. 3	29	do	do	2120
Feb. 4	29	do	do	2120
Feb. 10	Bled from heart	Titre, 1–640	do	2180
Feb. 11	29	1.0 cc. (i. v.)	do	2200
Feb. 16	29	1.5 cc. (i. v.)	do	2230
Feb. 17	29	do	do	2260
Feb. 18	29	do	do	2260
Feb. 24	Bled from ear	Titre, 1–640	do	2240
Feb. 25	Bled from heart	Titre, 1–640		

Normal serum obtained on the first day did not give a titre with the antigen.

Phenolized suspensions of the two gummosis types failed to give appreciable titres. The cauliflower organism proved toxic to the rabbit and care was taken in the next experiment in the injections of this antigen. The experiment was repeated without phenolizing the antigens. Living antigens were used.

Experiment II

Methods. The titres of the antisera obtained during the first experiment were not high enough to give any specificity to the sera. It was thought that the use of killed cultures reduced the possibility of obtaining high titres. In the second experiment the procedure was changed. Instead of using killed cultures throughout, living cultures were used during the second week. Three animals were used as before. Care was taken in giving the injections of the cauliflower organism. Young rabbits were used this time.

Results. The results in general were far more satisfactory than those of the first experiment, but the titres were not what could be expected. Only one of the antisera gave a titre over 2000. The cauliflower organism again caused necrosis of the rabbit's ear, but the rabbit responded and overcame the initial reaction. Charts 4, 5, and 6 show the paths of the reactions with the different rabbits.

Animal chart 4.

Rabbit 902.

Antigen, *Bacterium maculicolum*; 3 injections of phenolized; 3 injections of living.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Mar. 16	*B. maculicolum*	1.0 cc. (i. v.)	Excellent	2005
do	Bled from ear	No titre		
Mar. 17	*B. maculicolum*	1.0 cc. (i. v.) dead	Excellent	2000
Mar. 18	do	do	Necrosis of ear	1950
Mar. 19	———	———	No change	1960
Mar. 23	*B. maculicolum*	1.0 cc. (i. v.) living	Excellent	2060
Mar. 24	do	1.25 cc. do	do	2050
Mar. 25	do	1.50 cc. do	do	2140
Mar. 26	———	———	do	2100
Mar. 30	Bled from the ear	Titre, 1–2560	do	2190
Mar. 31	Bled from carotid	Titre, 1–2560		

Final titre, 1–2560.

The results clearly show that the injections of living organisms stepped up the titre appreciably. The titres are still too low for specific use. The experiment was then repeated, with living cultures only.

Experiment III

Methods. The titres obtained during the course of the second experiment indicated that the use of living cultures throughout would give the

Animal chart 5.
Rabbit 903.
Antigen, *P. cerasus* 28; 3 injections of phenolized, 3 injections of dead cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Mar. 16	28[a]	1.0 cc. (i. v.) dead	Excellent	1780
Mar. 17	28	do	do	1840
Mar. 18	28	do	do	1860
Mar. 19	....	.....	....	1910
Mar. 23	28	1.0 cc. (i. v.) living	do	1950
Mar. 24	28	1.25 cc. do	do	2000
Mar. 25	28	1.50 cc. do	do	2020
Mar. 26	....	.....	do	2040
Mar. 30	Bled from the ear	Titre, 1–1280	do	2100
Mar. 31	Bled from the carotid	Titre, 1–1280	do	2080

Final titre, 1–1280.
[a] Bled from the ear for normal blood titre. No titre.

Animal chart 6.
Rabbit 904.
Antigen, *P. cerasus* 29.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Mar. 16	29[a]	1.0 cc. (i. v.) dead	Excellent	1810
Mar. 17	29	do	do	1800
Mar. 18	29	do	do	1800
Mar. 19	....	.....	do	1900
Mar. 23	29	1.0 cc. living	do	1960
Mar. 24	29	1.25 cc. do	do	2000
Mar. 25	29	1.50 cc. do	do	1950
Mar. 26	....	.....	do	2060
Mar. 30	Bled from ear	Titre, 1–1280	do	2160
Mar. 31	Bled from carotid	Titre, 1–1280	do	.....

Final titre, 1–1280.
[a] Normal blood did not give a titre with the antigen.

expected results and that high specific serum titres could be obtained. In accordance with these observations the third experiment was conducted with living suspensions. The work was discontinued with the cauliflower antigen. Two rabbits were used for each of the gummosis organisms.

Results. Very good titres were secured during the course of the third experiment (Charts 7–10). Three of the rabbits responded with titres

above 5000, while the other rabbit gave a titre of only 1–2560. All were better than in the previous experiments. Animal 906 was carried through one more week than rabbit 905. At the end of the experiment the titre of rabbit 906 was not greater than that of 905. Small rabbits and large rabbits appear to react the same to this organism. At the end of the second week of injections the titres of the two rabbits were the same. Living cultures were used throughout and the results indicate that, in these two cases at least, living antigens have to be used in order that high-titre, potent sera can be obtained.

Animal chart 7.

Rabbit 905.

Antigen, *Ps. cerasus*, 28; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	28	1.0 cc. living (i. v.)	Excellent	2910
do	Bled from ear	Normal serum, no titre	do	2910
Apr. 7	28	1.0 cc. living (i. v.)	do	2870
Apr. 8	28	do	do	2940
Apr. 13	28	1.5 cc. living (i. v.)	do	3020
Apr. 14	28	do	do	2960
Apr. 15	28	do	do	3080
Apr. 20	Bled from ear	Titre, 1–5120	do	3300
Apr. 23	Bled from carotid	Titre, 1–5000	do	3360

Final titre, 1–5000.

Agglutination Experiments

Having produced these excellent titres, the two types were then subjected to cross agglutination experiments. As the organisms were so closely related, it was thought that they would cross agglutinate to some degree. Strange to say the organisms proved very specific: no cross agglutination was noted. *Pseudomonas cerasus* 28 and 29 are apparently distinct types causing the same disease. They did not cross agglutinate with the cauliflower organism, *B. maculicolum*. *Bacillus typhosus, Bacillus coli* and *Staphylococcus aureus* were subjected to the tests and they also failed to show any cross agglutination with the antisera of *Ps. cerasus*.

Methods. The agglutination titrations were carried out under the standard method as outlined in Kolmer's text. Agar streak cultures, 24 hours old, were suspended in physiological salt solution so that the suspension had a turbidity equal to that of a standard tube which should contain 2,000,000,000 units per cc. The writer used a standard furnished by Dr.

Animal chart 8.
Rabbit 906.
Antigen, *Ps. cerasus*, 28; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	28	1.0 cc. (i. v.) living	Excellent	1820
do	Bled from ear	Normal blood, no titre	do	1820
Apr. 7	28	1.0 cc. (i. v.) living	do	1740
Apr. 8	28	1.5 cc. (i. v.) living	do	1780
Apr. 13	28	do	do	1940
Apr. 14	28	do	do	1860
Apr. 15	28	do	do	1880
Apr. 20	Bled from ear	Titre, 1–5120	do	2050
Apr. 23	28	2.0 cc. (i. v.) living	do	2150
Apr. 24	.....	.....	do	2080
Apr. 26	.....	.....	do	2200
Apr. 28	.....	.....	do	2020
May 1	Bled from ear	Titre, 1–5120	do	2150
May 4	Bled from carotid	Titre, 1–5120	do	2320

Final titre, 1–5120.

Animal chart 9.
Rabbit 907.
Antigen, *Ps. cerasus*, 29; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	Bled from ear	Titre, 0	Excellent	1910
do	29	1.0 cc. (i. v.) living	do	1910
Apr. 7	29	do	do	1910
Apr. 8	29	1.5 cc. (i. v.) living	do	2020
Apr. 13	29	do	do	2060
Apr. 14	29	do	do	2000
Apr. 15	29	do	do	2060
Apr. 20	Bled from ear	Titre, 1–5120	do	2060
Apr. 23	Bled from carotid	Titre, 1–5120	do	2080

Final titre, 1–5120.

Alvarez. The standard was duplicated and used for the remainder of the experiment. One half of one cc. standard suspension was added to each 0.5 cc. of diluted serum, in saline. The mixture was allowed to stand in a water bath for two hours at 37° C. Readings were made each hour. At

Animal chart 10.
Rabbit 908.
Antigen, *Ps. cerasus*, 29; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	Bled from ear	Titre, 0	Excellent	2150
do	29	1.0 cc. (i. v.) living	do	2150
Apr. 7	29	do	do	2050
Apr. 8	29	1.5 cc. (i. v.) living	do	2160
Apr. 13	29	do	do	2160
Apr. 14	29	do	do	2200
Apr. 15	29	do	do	2220
Apr. 20	Bled from ear	Titre, 1–5120	do	2280
Apr. 23	29	2.0 cc. (i. v.) living	do	2320
Apr. 24	—	—	do	2250
Apr. 26	—	—	do	2400
Apr. 28	—	—	do	2210
May 1	Bled from ear	Titre, 1–2560	do	2360
May 4	Bled from carotid	Titre, 1–2560	do	2460

Final titre, 1–2560.

Titre dropped off after last injection was given. The reason for this is not apparent since this phenomenon did not occur in the rabbit so treated with antigen 28. The rabbit lost weight rapidly after the last injection and possibly some of the agglutinins were used up in the defense.

the end of the two hours the tubes were placed in the ice-box and then read the following morning. Saline plus antigen and serum were used throughout the experiment as checks.

Agglutination tests were made with the normal sera and immunized sera. Cross agglutination was tested with the immunized sera of the three antigens.

Results. In tables 1 and 2 the relation of the antigens to the homologous sera and to the normal sera are noted. Table 3 gives the cross agglutinating values of the different sera.

DISCUSSION AND CONCLUSIONS

The production of satisfactory agglutinating antisera against phytopathogenic bacteria appears to be as simple as against animal parasitic bacteria. Apparently the phytopathogenic forms differ greatly in their power to produce antibodies in the sera of rabbits. This power seems to be correlated with the toxic products that are elaborated.

TABLE 1.—*The results of agglutination tests with normal sera of rabbits 905, 906, 907, and 908 and the antigens of Pseudomonas cerasus 28 and 29. Tests made April 8, 1925.*

Serum and antigen	Dilutions									Time and temperature
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Ck.	
Serum 905	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen 28	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.
Serum 906	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen 28	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.
Serum 907	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen 29	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.
Serum 908	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen 29	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.

TABLE 2.—*The results of agglutination tests with the antisera of rabbits 905, 906, 907, and 908 and the antigens of Pseudomonas cerasus 28 and 29*

Antiserum and antigen	Dilutions								Time and temperature
	1/320	1/640	1/1280	1/2560	1/5120	1/10000	Ck.	Ck.	
Antiserum 905	+	+	+	+	–	–	–	–	1 hr., 37° C.
+	+	+	+	+	+	–	–	–	2 hrs., 37° C.
Antigen 28	+	+	+	+	+	–	–	–	18 hrs., 8° C.
Antiserum 906	+	+	+	+	–	–	–	–	1 hr., 37° C.
+	+	+	+	+	+	–	–	–	2 hrs., 37° C.
Antigen 28	+	+	+	+	+	–	–	–	18 hrs., 8° C.
Antiserum 907	+	+	+	+	–	–	–	–	1 hr., 37° C.
+	+	+	+	+	+	–	–	–	2 hrs., 37° C.
Antigen 29	+	+	+	+	+	–	–	–	18 hrs., 8° C.
Antiserum 908	+	+	+	–	–	–	–	–	1 hr., 37° C.
+	+	+	+	+	–	–	–	–	2 hrs., 37° C.
Antigen 29	+	+	+	+	–	–	–	–	18 hrs., 8° C.

From the results of the experiments, it appears that, in the case of some phytopathogenic bacteria, production of suitable antisera can only be accomplished by the injection of living organisms in fairly large doses. In the

TABLE 3.—*The results of direct and cross agglutination tests with antisera of rabbits 902, 905, and 907, the antigens of Pseudomonas cerasus 28 and 29, and the antigen of Bacterium maculicolum*

Antiserum and antigen	Dilutions									Time and temperature
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Ck.	
Antiserum 905	+	+	+	+	+	+	+	+	–	1 hr., 37° C.
+	+	+	+	+	+	+	+	+	–	2 hrs., 37° C.
Antigen 28	+	+	+	+	+	+	+	+	–	18 hrs., 8° C.
Antiserum 907	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen 28	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.
Antiserum 907	+	+	+	+	+	+	+	+	–	1 hr., 37° C.
+	+	+	+	+	+	+	+	+	–	2 hrs., 37° C.
Antigen 29	+	+	+	+	+	+	+	+	–	18 hrs., 8° C.
Antiserum 905	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen 29	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.
Antiserum 902	+	+	+	+	+	+	+	+	–	1 hr., 37° C.
+	+	+	+	+	+	+	+	+	–	2 hrs., 37° C.
Antigen *B. maculicolum*	+	+	+	+	+	+	+	+	–	18 hrs., 8° C.
Antiserum 905	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen *B. maculicolum*	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.
Antiserum 907	–	–	–	–	–	–	–	–	–	1 hr., 37° C.
+	–	–	–	–	–	–	–	–	–	2 hrs., 37° C.
Antigen *B. maculicolum*	–	–	–	–	–	–	–	–	–	18 hrs., 8° C.

case of the species that produce toxic products, extremely potent antisera can be hoped for. Titres against the ordinary forms cannot be expected to go higher than 1–10,000.

The writer feels that more work of this nature should be undertaken by plant as well as animal bacteriologists. Serological applications will go a long way in placing plant bacteriology on a sound basis. Too many of our phytopathogenic species are described without any effort to compare with known forms. Many of our pathogenic species closely resemble each other not only in the disease manifestation but in their morphological and biochemical characteristics. Antisera can be used not only in identification work but in studying epidemiology. Little is known about the dissemination, persistence and many other phases of our pathogenic forms. Possibly the greatest drawback to the plant bacteriological science has been the lack

of some means of identification without resort to inoculation experiments. On the successful inoculation rests the fate of many isolated organisms. Successful inoculations are rather rare in comparison with unsuccessful. Too much depends upon the condition of the host and the parasite as to whether an inoculation will be successful or not. The use of a highly potent antiserum should eliminate a great deal of the inoculation work and inconsistency in results.

SUMMARY

1. The literature on the production of antisera against phytopathogenic bacteria is discussed.

2. Experiments upon the production of antisera against three phytopathogenic forms, *Pseudomonas cerasus* var. 28, *Ps. cerasus* var. 29 (two bacterial gummosis types), and *Bacterium maculicolum*, an organism causing the spot disease of cauliflower, were conducted.

3. Potent antisera were produced against the three forms.

4. Living antigens gave the best results when injected intravenously.

5. Cross agglutination was not encountered.

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NOTES ON THE CROWNGALL SITUATION IN ENGLAND, FRANCE AND HOLLAND[1]

A. J. Riker

A survey of the crowngall situation in England, France, and Holland was made in the fall of 1926 in order to determine whether or not any correlation existed between the situation in these countries and that in the United States. The survey was undertaken in connection with recent work in the United States which has shown that a considerable number of the enlargements formed on certain plants, particularly nursery apple trees, were caused by other agencies than *Bacterium tumefaciens* Smith and Town. Miss Brown (1) has shown that the aerial stem tumors on apple, which were sometimes called burr knots, were not crowngall. Riker and Keitt (6), Melhus (3), and Muncie (4) reached the conclusion that, while true crowngall was widespread, a very considerable percentage of the enlargements at the crown of nursery apple trees followed mechanical injuries or were incident to the grafting process. In Europe also, Wormald and Grubb (8) have concluded that the crowngall organism was not the only factor in the development of these enlargements. In view of this revision of the crowngall problem it seemed desirable to correlate the actual situation and the work which was being done on this subject both in America and Europe.

The methods employed in making this survey were similar to those described by Riker and Keitt (6) for the United States. Representative nurseries in England, France, and Holland in which apple trees and other susceptible stock were being grown were visited in the fall of 1926 and the plants examined as they were dug for shipment. When it was impractical to examine very large numbers of individual plants the percentages were secured from counts on several hundred plants taken at random. As the writer did not have facilities for making extensive cultural examinations of the material, the diagnosis was dependent upon the characteristics of crowngall and of wound overgrowth as described by Riker and Keitt (6). However, as they have shown, these characters are sufficiently distinctive so that a very high degree of accuracy may be secured.

In consideration of the results of this survey and in correlating them with the conditions in the North-central and Northeastern United States it is important to hold in mind several important factors which are different

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

in certain sections of Europe from those in the United States. Notable among these are differences in environmental conditions, in the varieties of plants cultivated, and in the cultural practices employed.

The environmental conditions in England, Holland, and Northwestern France are in some respects quite unlike those in North-central and North-eastern United States. The latitude in this section of Europe provides for a very much longer day in the summer season when the plants are making the major portion of their growth and for very short days in the winter when the plants are dormant. At the same time the temperature is moderated so that burning hot days in the summer and very severe frost in the winter, which always occur in this section of the United States, are practically unknown. The moisture is supplied in a particularly favorable way. While the actual precipitation may, in this section of Europe, not be any greater, the average relative humidity of the air is considerably above that in this section of the United States, and the actual rainfall is distributed in frequent gentle showers which tend to keep the ground relatively moist. These and other factors combine to make the growing season considerably longer. At the same time the comparatively cool temperature appears to be influential in preventing the very rapid type of plant growth which is common during the spring and early summer in this section of America. Of course all these factors which influence plant growth are also important in the development of disease (see Jones *et al.* 2). The influence of certain environmental factors on the development of crowngall has been described by Riker (5).

The varieties of susceptible plants grown in this section of Europe are different in many cases from those grown in this section of the United States. It is quite apparent that there is a much greater requirement for fruit trees of small size in Europe. The comparatively high price of land, the small portions held by one individual, and the comparatively cheap labor have developed a very intensive cultivation. It appears that this situation is largely responsible for the great popularity, in some regions, of Doucin and Paradise apple stock because the trees are more or less dwarfed and bear fruit at an earlier age. At the same time the climate in this section of Europe, with its comparatively mild winters and cool summers, favors the propagation of some varieties which would not live under certain American conditions. While exact information on the relative susceptibility to crowngall of different types of fruit trees is lacking, especially as it has been shown by several workers, as mentioned above, that many of the enlargements formerly called crowngall are not due to *Bact. tumefaciens*; nevertheless it is an important factor to be considered.

The method of propagation of fruit trees has been found to be very important in the incidence of wound overgrowth. In North-central United

States, of apple trees of the Wealthy variety which were propagated by piece root grafts, there was often 35 per cent with wound overgrowths (6), while the same variety which was propagated in adjacent rows by budding showed less than 3 per cent. Inasmuch as the piece-root graft for the propagation of apple trees, which is very common in the North-central United States, is quite unusual in Europe, it is not to be expected that European budded stock should show so much wound overgrowth.

Certain enlargements which are characteristic of particular varieties have not been mentioned in this survey. No record was taken of the presence of burr-knots which appear almost always on Paradise, Doucin, and certain other stocks. Although they were at one time considered to be crowngall, it has been shown by Brown (1) that they are not due to *Bact. tumefaciens* and by Swingle (7) that they are characteristic of certain varieties of stock.

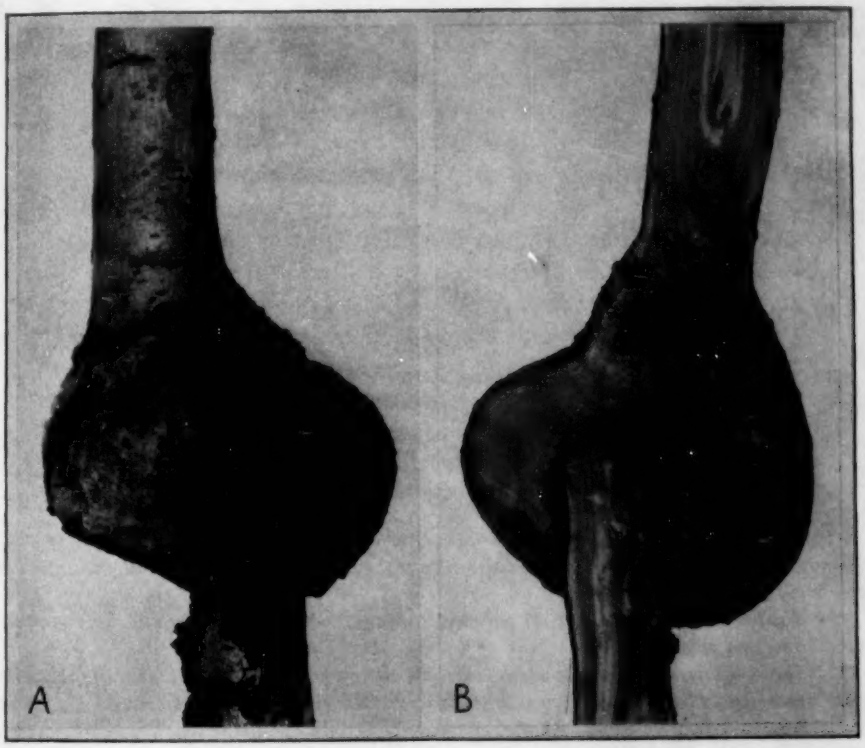

FIG. 1.—Enlargements formed in four years at the unions where vigorous apple cions were budded on dwarfing stocks. A. Surface. B. Section. ×2/5. These specimens were provided by M. B. Crane and photographed by H. C. Osterstock, of the John Innes Horticultural Institution.

TABLE 1.—*Summary of the findings by a survey in England, France, and Holland, during the fall of 1926 for crowngall and wound overgrowth on nursery stock*

Nursery	Kind of stock	Wound overgrowth[a] (per cent)	Crown-gall (per cent)	Nursery	Kind of stock	Wound overgrowth[a] (per cent)	Crown-gall (per cent)
			England				
A	Apple	5	0	A con.	Raspberry	0	10[b]
	do. seedling	1	0	B	Apple	6	trace
	Peach	0	0		Cherry	0	0
	Pear	trace	trace		Plum	0	0
	Plum	0	0		Raspberry	0	4
			France				
A	Apple	10	0	D con.	Pear	0	0
	do. seedling	trace	0	E	Apple	0	0
	Pear	10	0		Cherry	0	0
	Rose	0	0		Pear	0	0
B	Apple	8	0		Rose	0	0
	do. seedling	trace	0	F	Apple	0	0
	Cherry	0	0		do. seedling	0	0
	Quince	1	0		Cherry	0	0
C	Apple	10	0		Pear	0	0
	Pear	trace	0	G	Apple	0	0
	Plum	0	0		Pear	0	0
D	Apple	trace	0		Rose	0	0
	do. seedling	0	0	H	Apple	0	0
	Cherry	0	0		Pear	0	0
	Peach	0	0		Plum	0	0
			Holland				
A	Apple	8	0	E	Rose	1/10	0
	Cherry	12	0	F	Apple	1	0
	Rose	trace	0		Rose	trace	0
B	Rose	1/10	trace[c]	G	Apple seedling	0	0
C	Apple	70	0		do.	trace[e]	0
	Rose	trace	0	H	Apple	4	10
D	Apple	2	6		Pear	5	15
	Pear	60	10[d]				

[a] This class includes excess callus, wound overgrowths, and non-infectious hairy-root. However, burr-knots, which are normal on Paradise, Doucin, and certain other stocks are not included.

[b] One lot of raspberries had 40 per cent crowngall while three other lots were entirely free from this disease.

[c] These figures are based on examination of representative samples of 250,000 Monetti rose stocks intended for shipment to America. No crowngall was found in the stock being prepared for shipment. In the pile of rejects only one plant was found which bore an enlargement resembling crowngall.

[d] The diagnosis is doubtful. A number of the plants bore enlargements which were typical of neither wound overgrowth nor crowngall. Similar enlargements have been observed on pears grown on drained swamp land in Michigan.

[e] Callus and wound overgrowths were found only on transplanted seedlings.

Likewise no record was taken of such enlargements as those which very commonly appear at the union of apple trees budded on dwarfing stock (Fig. 1). The presence of these enlargements on such trees and their nonparasitic nature is common information in regions where this type of stock is grown. Their development is ordinarily explained on the basis of lack of congeniality between stock and cion. Ordinarily the budding in such cases is done sufficiently high so that these enlargements are several inches above the soil line. This is necessary because, where the enlargement touches the soil, it sends out a large number of roots (one type of hairy-root), and such a tree, becoming established on roots from the cion, is no longer dwarfed by the stock. It appears that the question of congeniality between stock and cion is important and should be considered in efforts to eliminate wound overgrowths from piece-root apple grafts.

A summary of the findings of this survey is given in table 1. In England and Holland, crowngall was an important factor in only three or four places. In each of these cases the size of the affected planting was so small that the owner was not concerned about any loss. In each of these instances also the owner never sold any of his plants in the United States. In Northwestern France no crowngall was observed. Both the nurserymen and the nursery inspectors are familiar with the disease and report that, while they find it from time to time, it is never of any serious consequence. Wound overgrowth was found in limited quantities in all the countries visited. When allowance is made for differences in climate, varieties cultivated, horticultural practices, and so forth, it appears that the crowngall and wound overgrowth situations in these European sections are very similar indeed to those in the North-central and Northeastern United States. In all these regions it appears that the malformations encountered in the underground parts of the nursery stock examined were predominantly callus, hairy-root, or wound overgrowths. Although it seems that infections by *Bact. tumefaciens* appear from time to time in all regions visited, the economic importance of true crowngall, except in isolated cases, is commonly very slight.

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EXPERIMENTS ON THE CONTROL OF BARLEY STRIPE[1]

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INTRODUCTION

Attempts have been made recently to improve methods of treating barley seed for controlling barley stripe, *Helminthosporium gramineum* Rabh. The modified hot water method is effective but cumbersome, and the long-time soak in formaldehyde is not always effective. There also is some evidence that formaldehyde sometimes injures barley seed. Leukel, Dickson, and Johnson[2] have shown that some of the organic mercury compounds, including certain dusts, are quite effective against stripe. For several years the writer has been testing the effectiveness of these compounds, and the results are presented in this paper.

The control of barley stripe is assuming greater importance in Minnesota, as the amount of the disease seems to be increasing. This may be due to the fact that some of the varieties recently produced and distributed in Minnesota are quite susceptible to the disease. Minsturdi, Minn. 439, which is replacing to some extent Manchuria, Minn. 184, is quite susceptible. From 5 to 40 per cent of the plants of this variety usually are infected. There is some evidence, also, that the disease is increasing in certain other varieties grown in the State, especially Svansota, Minn. 440. This may be because of the gradual accumulation of inoculum due to the growing of susceptible varieties, or because of the increase of a more virulent strain of the pathogene. Johnson's work[3] indicates that there may be several physiologic forms of *H. gramineum* in the State. Whatever the cause, it is perfectly evident that the disease is becoming more destructive, and it is imperative that control measures be simplified and improved.

The literature on the control of barley stripe has been reviewed thoroughly by Leukel, Dickson, and Johnson, and will therefore not be reviewed in this paper.

[1] Published with the approval of the Director as paper No. 740 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Leukel, R. W., James G. Dickson and A. G. Johnson. Experiments with dusts for controlling stripe disease of barley. Phytopath. 17: 175–179. 1927.

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MATERIALS AND METHODS

Both liquid and dust fungicides were used in the tests for the control of the stripe disease of barley. The chemicals used and the names of the manufacturers are as follows:

Bayer Dust Uspulun Höchst	Bayer Company, Inc., New York City.
Semesan Semesan Dust Du Pont No. 12 K–I–A K–I–B	E. I. du Pont de Nemours and Campany, Wilmington, Delaware.
Germisan S. A. F. No. 225	Saccharin-Fabrik, Aktiengesellschaft, vorm. Fahlberg, List and company, Magdeburg, Südost, Germany.
Wa Wa Dust	Chicago Process Company, Chicago, Illinois.
Coppercarb	Pittsburgh Plate Glass Company, Corona Chemical Division, Milwaukee, Wisconsin.
Kolo Dust	Niagara Sprayer Company, Middleport, N. Y.
Formaldehyde	Roessler and Hasslacher Chemical Company, Perth Amboy, New Jersey.

Minsturdi barley (Minn. 439) was soaked in Uspulun, 0.25 per cent solution; in Germisan, 0.25 per cent solution; Semesan, 0.3 per cent solution; and formaldehyde, 1–320. The periods of soak were one, two, and three hours at ordinary temperatures, and one half, one, and two hours at 45° C. Formaldehyde was also used as a dip, and the seed subsequently covered for five hours. Numerous plots were sown with untreated seed for comparison. The dust fungicides were used at the rate of 4 ounces to a bushel of seed. The dust and seed were shaken together in a 2-liter Erlenmeyer flask until the seed was thoroughly coated with a fine layer of dust. All of the liquid treatments were made so that the seed was thoroughly dry three days before planting. In 1925 and 1926, when yield data were taken, the seed was sown in triplicate eighteen foot rows in plots which were replicated three times. Counts on individual culms and yield data were taken from the center row of the triplicate plots. The approximate number of culms per row was between four hundred and four hundred and fifty. In 1924 and 1927 the seed was sown in individual rod rows in series of three and four, respectively.

TABLE 1.—*Effect of liquid fungicides on percentage of stripe (1924–1927) and on yield (1925–1926) of Minsturdi barley at University Farm, St. Paul, Minnesota*

Treatment		Acre yields			Percentage of culms infected				
		1925	1926	Average	1924	1925	1926	1927	Average
Uspulun, 0.25 per cent solution	1 hour cold	66.0	62.5	64.3	tr.[a]	0.8	0.6	0.5	0.5
	2 hours cold	70.7	61.8	66.3	tr.	tr.	0.0	—	tr.
	3 hours cold	69.6	61.7	65.7	0.0	tr.	0.0	—	tr.
	½ hour at 45° C.	79.1	57.5	68.3	0.8	tr.	0.5	—	0.4
	1 hour at 45° C.	72.7	67.3	70.0	0.0	tr.	0.0	—	tr.
	2 hours at 45° C.	67.7	61.4	64.6	0.0	0.0	0.0	—	0.0
Germisan, 0.25 per cent solution	1 hour cold	71.3	58.6	65.0	2.8	0.0	2.2	tr.	1.3
	2 hours cold	77.0	61.0	69.0	1.8	0.0	0.7	—	0.8
	3 hours cold	72.3	62.3	67.3	0.8	0.0	1.0	—	0.6
	½ hour at 45° C.	75.9	59.7	67.8	0.7	0.0	0.8	—	0.5
	1 hour at 45° C.	71.3	64.3	67.8	tr.	0.0	0.4	—	0.1
	2 hours at 45° C.	65.2	61.2	63.2	0.0	0.0	0.3	—	0.1
Semesan, 0.3 per cent solution	1 hour cold	75.9	63.7	69.8	tr.	0.7	0.5	1.0	0.6
	2 hours cold	75.3	68.5	71.9	0.5	0.5	0.3	—	0.4
	3 hours cold	74.7	60.6	67.7	tr.	tr.	0.4	—	0.1
	½ hour at 45° C.	72.5	58.9	65.7	0.0	tr.	0.5	—	0.2
	1 hour at 45° C.	71.6	63.7	67.7	0.0	tr.	0.5	—	0.2
	2 hours at 45° C.	77.2	61.5	69.4	0.0	0.0	0.0	—	0.0
Formaldehyde, 1–320	Dip, cover 5 hours	63.9	55.8	59.9	2.5	10.6	6.6	—	6.6
	1 hour cold	61.4	69.9	65.7	0.3	4.2	2.3	6.6	3.4
	2 hours cold	65.7	64.8	65.3	0.4	2.5	1.5	—	1.5
	3 hours cold	67.7	58.7	63.2	0.8	2.7	1.8	—	1.8
	½ hour at 45° C.	56.2	65.6	60.9	0.3	4.2	2.3	—	2.3
	1 hour at 45° C.	54.9	70.5	62.7	0.0	3.1	1.6	—	1.6
	2 hours at 45° C.	25.2	56.6	40.9	0.0	2.0	1.0	—	1.0
Control, tap water	Dry	64.0	56.2	60.1	13.2	11.2	10.1	6.7	10.3
	1 hour cold	66.2	67.7	62.0	—	10.5	11.4	6.6	9.5
	2 hours cold	65.1	57.8	61.5	—	9.8	10.9	—	10.4
	3 hours cold	67.6	58.0	62.8	—	9.1	9.2	—	9.2
	½ hour at 45° C.	70.4	63.6	67.0	—	4.4	5.7	—	5.1
	1 hour at 45° C.	71.5	58.2	64.9	—	3.5	2.1	—	2.8
	2 hours at 45° C.	75.8	64.7	70.3	—	1.8	3.6	—	2.7

[a] tr. = trace of infection.

RESULTS

The results of treating barley with liquid fungicides to control barley stripe and the yield data are presented in table 1.

It was found that the effectiveness of the liquid fungicides used depended somewhat on the temperature and length of soak. Solutions of Uspulun and Semesan reduced barley stripe to 1 per cent or less for a period of years when the seed was treated for one hour at room temperature. In the corresponding control plot an average of 9.5 per cent of stripe developed. When the period of soak was increased to two and three hours,

Uspulun eliminated the stripe or reduced it to a trace, while Semesan reduced it to 0.5 per cent or less. An average of 10.4 and 9.2 per cent of stripe developed in the plots sown with seed soaked in tap water for two and three hours, respectively. The results obtained with Germisan were conflicting. In 1925 it eliminated the stripe under all the conditions tried, and in 1927 it reduced stripe to a trace. In 1924 and 1926, however, Germisan was not so effective as Uspulun or Semesan when the seed was soaked in the solution at ordinary temperatures. One-half hour treatment in Uspulun, Germisan, and Semesan, at 45° C., reduced the stripe to less than 1 per cent as compared with an average of 5.1 per cent of stripe in the control plot. Germisan and formaldehyde caused delayed heading of the grain in 1924 and 1925 when the period of soak at 45° C. was extended to one and two hours. Formaldehyde dip reduced the amount of stripe but not sufficiently for practical purposes. When used at room temperature for the various periods of soak it controlled the stripe in 1924. In other years formaldehyde reduced the stripe but was not so effective as the organic mercury compounds tested. Hot formaldehyde caused marked injury to the stand in 1924.

In no instance did the plants of individual plots emerge from the soil early enough to warrant the statement that the fungicide stimulated germination of the seed. Yield data were taken in 1925 and 1926. In comparing the yield data in the individual years, there were apparently no significant differences in the yields obtained from plots treated with the organic mercury compounds. Formaldehyde reduced the yields in 1925 but had no apparent effect on them in 1926.

The results of the dust treatments are given in table 2. Four of the ten dusts used controlled stripe satisfactorily. K–I–A and K–I–B eliminated the stripe in all four series, while Du Pont No. 12, S. A. F. No. 225, and Wa Wa Dust reduced it to an average of 0.4 per cent or less. These dusts, therefore, appear very promising, although their effect on yields was not determined. However, K–I–A and K–I–B were used by the writer for treating oats and hulless barley against smut and did not decrease yields. Bayer Dust, Semesan Dust, and Höchst reduced the stripe to less than 2 per cent, while the Coppercarb and Kolo Dust were ineffective in controlling the stripe.

DISCUSSION

It is evident that several of the organic mercury compounds, notably Uspulun, Semesan, and Germisan, when used in solution, are preferable to formaldehyde for controlling barley stripe. All of these fungicides controlled stripe quite effectively when used in cold, as well as in hot, solution. In addition, the yields were better than those obtained from untreated

TABLE 2.—*The effect of chemical dusts on the percentage of stripe in Minsturdi barley at University Farm, St. Paul, Minnesota, in 1927*

Fungicide[a]	Percentage of infected culms				
	Series				Average
	A	B	C	D	
Coppercarb	4.2	4.4	5.2	4.8	4.7
Kolo Dust	5.3	5.3	4.8	7.8	5.8
Bayer Dust	2.3	2.0	0.7	1.2	1.6
Semesan Dust	2.0	tr.	tr.	2.3	1.1
Untreated	7.0	5.2	7.4	7.2	6.7
Wa Wa Dust	tr.[b]	0.7	0.0	tr.	0.2
Höchst	2.9	0.7	1.2	2.2	1.8
S. A. F. No. 225	tr.	0.0	0.7	tr.	0.2
Du Pont No. 12	tr.	0.0	tr.	1.5	0.4
Untreated	6.9	7.2	7.5	7.5	7.3
K–I–A	0.0	0.0	0.0	0.0	0.0
K–I–B	0.0	0.0	0.0	0.0	0.0

[a] Applied at the rate of 4 oz. a bushel of seed.

[b] tr. = trace of infection.

grain, although there was no evidence that the fungicides had any special stimulatory effect. Formaldehyde was not so effective as these organic mercury compounds and also had a tendency in some cases to reduce yields. It seems safe to conclude, therefore, that the organic mercury compounds are preferable to formaldehyde. And they certainly are preferable to the modified hot-water treatment because of the saving of time and labor.

Certain of the dust fungicides were surprisingly effective, but their effect on yields is not yet known. However, the writer has treated oats and hulless barley with some of these dusts, and no deleterious effects were noticed. It seems quite probable, therefore, that they can be used effectively for controlling stripe. The results with the dusts indicate that the organism causing stripe probably is often under the hull of the barley and not inside of the seed coats. This possibility is strengthened by the fact that Johnson[4] showed that barley became infected readily if the de-hulled seed was inoculated. While more experiments must be made, and yield data taken, it seems probable that chemical dusts eventually will be used successfully for the control of stripe.

SUMMARY

1. Four liquid fungicides, used at different temperatures and periods of soak, and ten dust fungicides were tested for their effectiveness in controlling barley stripe.

[4] Johnson, *loc. cit.*

2. The effectiveness of the liquid fungicides depends on the temperature of the solution and the period of soak. Uspulun and Semesan were the most satisfactory fungicides for the control of stripe when the seed was soaked for one hour at room temperature. Uspulun, Germisan, and Semesan were about equally effective when the seed was soaked at 45° C. for one-half hour.

3. Yields were increased somewhat as a result of treating seed with the organic mercury compounds, although hot Germisan caused a delay in heading. Hot formaldehyde reduced yields in 1925, and also caused a delay in heading.

4. K–I–A and K–I–B dusts eliminated stripe entirely, while Du Pont dust No. 12, S. A. F. dust No. 225, and Wa Wa Dust were almost as effective. Coppercarb and Kolo Dust were ineffective in controlling stripe.

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CLUB ROOT IN RELATION TO SOIL ALKALINITY

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The effect of alkalinity on the presence of *Plasmodiophora brassicae* Wor. in the soil was noted even before the organism was described and named. It was found that an application of lime or wood ashes decreased the amount of disease, or controlled it entirely. This was before the time when apparatus was available for determining fairly accurately the acidity of the soil; so many workers merely guessed at the amount of lime to use, as is shown by some of the following recommendations:

256 bu. lime or 10–12 cu. yds. turf ashes an acre; Farquharson (3), 1831
14–16 tons lime an acre; Hunter (7), 1857
700 lbs. lime an acre (in drill with seed); Somerville (12), 1894
2 tons lime an acre; Voelcker (14), 1894
90 bushels lime an acre; Stewart (13), 1895
37.5–150 bushels lime an acre; Halsted (4), 1896
400 liters lime an acre; Mahieu-Sanson (9), 1897
6–8 tons lime an acre; Hawk (5), 1898
75–150 bushels lime an acre; Jones (8), 1898

Later writers, probably quoting Ravn (11), stated that the critical point at which the organism ceased to cause infection was at or slightly above the neutral point, which probably compared roughly with our present day designation of pH 7.00.

Only one writer seems to have made hydrogen-ion determinations of soils that were free of club-root, and of those in which it was abundant. Atkins (1) in Ireland found a field divided by a small stream of water. On one side of the stream, where the soil tested pH 6.6, *Plasmodiophora* caused much injury. On the other side there was no disease and the soil tested pH 6.7 or slightly above. Atkins realized that the information was not sufficient to justify drawing definite conclusions. Judging from the results of other workers, however, he felt that the above figures were not far wrong.

The writer undertook experiments to determine, if possible, the pH range in which the pathogene lives and is able to cause infection. The purpose of these tests was to determine the amount of hydrated lime required to adjust the pH values of the soil to a point at which the development of the club root organism would be inhibited. It soon was found, however, that the pH values increased rapidly after lime was added but then gradu-

ally diminished, so that the plants in any one series were subjected to a relatively wide range of hydrogen-ion concentration. It was found, also, that the reaction of the soil in pot cultures in the greenhouse fluctuated even more widely than did that of soil in the field.

The only method left for determining the relationship of club-root and soil alkalinity was to conduct numerous experiments in which the determinations were made at frequent intervals, and then plot a curve from all the averages thus procured. For the experiments, seven plots were staked off in the field with buffer plots at each end. The second and fifth plots were checks; the rest were treated with hydrated lime or with sulfur, at the following rates: Plot 1, 1,000 lbs. lime an acre; plot 3, 2,000 lbs. lime an acre; plot 4, 640 lbs. sulfur an acre; plot 6, 3,000 lbs. lime an acre; plot 7, 320 lbs. sulfur an acre. During each planting approximately 1,600 cabbage plants were grown in each plot.

For the inoculum, clubbed roots were ground in a meat grinder, and the watery pulp scattered along the furrow in which the seeds were dropped. When the plants were large enough so that club-root could be detected, half

TABLE 1.—*The effect of applications of lime and sulfur on the pH of the soil and the development of club-root on cabbage. Field experiments at New Brunswick, New Jersey*

Plot no.	Treatment	Percentage disease, July 6	pH, July 6	Percentage disease, July 26	pH, July 26	Percentage disease, Aug. 31	pH, Aug. 31
1	1000 lbs. lime an acre	30.8 ± 3.47	5.78	74.0 ± 3.5	5.93	85.7 ± 0.96	6.22
2	Check—no treatment	40.9 ± 4.7	5.20	89.0 ± 2.18	5.20	93.32 ± 3.44	5.51 6.08
3	2000 lbs. lime an acre	31.5 ± 4.13	6.07	72.2 ± 4.98	6.35 6.52	81.22 ± 4.05	6.56 6.85
4	640 lbs. sulfur an acre	28.0 ± 4.54	5.12	48.9 ± 0.22	5.14	80.0 few plants alive	4.37 4.94
5	Check—no treatment	43.7 ± 4.82	5.48	83.1 ± 2.43	5.56	93.78 ± 1.93	5.29 5.29
6	3000 lbs. lime an acre	28.8 ± 4.01	6.61	42.5 ± 6.59	6.68	3.2 ± 0.78	7.09 7.24 7.36
7	320 lbs. sulfur an acre	28.6 ± 6.53	5.04	45.0 ± 6.92	4.83	91.0 few plants alive	5.44 5.70

of the plants were pulled up and the percentage of disease determined. The remaining plants, now having more space in which to grow, were left 20 days longer. The second crop was planted as soon as harvesting of the first was completed. Inoculum was again strewn in the furrow. The results of the two plantings are given in table 1.

The first determinations of the pH values were made by the colorimetric method, and are not so accurate as are later ones made with the potentiometer.

It can readily be seen from the table that 1,000 and 2,000 lbs. of hydrated lime an acre on soil with a pH only slightly above 5.00 had very little effect on the percentage of club-root. But when 3,000 lbs. were applied two months before planting so that the soil was slightly above pH 7.00, the amount of disease was reduced to 3.2 per cent.

In the greenhouse, where similar work was conducted by using inoculated soil in two-gallon earthen jars the percentage of disease in soil that attained pH 6.20 to 6.85 was never so high as it was in the field; but in other ways the results were quite comparable. This was true especially of the more alkaline soil.

Twenty-two jars were used, and six successive crops were grown. An attempt was made to have a fairly uniform gradation of soil reaction in each experiment, from soil too alkaline for the growth of the slime mold to that in which club-root developed at a maximum rate. Hydrogen-ion determinations were made by means of a potentiometer each week from the time the seed was sown until the plants were harvested. The results of these tests are summarized in table 2.

The results are shown more plainly in graph 1.

Apparently the point at which the growth of *Plasmodiophora brassicae*

TABLE 2.—*The effect of hydrogen-ion concentration of soil on the development of club-root of cabbage. Greenhouse experiments at New Brunswick, New Jersey*

No. of individual jars	Range of disease percentages	Average percentage disease	Minimum pH	Maximum pH	Average pH
14	0	0	7.52	8.00	7.71
4	Less than 2	1.2	6.65	7.33	7.30
24	2 to 10	3.8	6.20	6.95	6.61
14	11 to 20	15.6	5.80	6.62	6.20
4	21 to 30	25.6	5.89	6.46	6.20
6	31 to 40	32.5	5.72	6.41	6.05
10	41 to 50	44.5	5.47	5.94	5.71
12	51 to 100	84.6	5.46	6.00	5.73

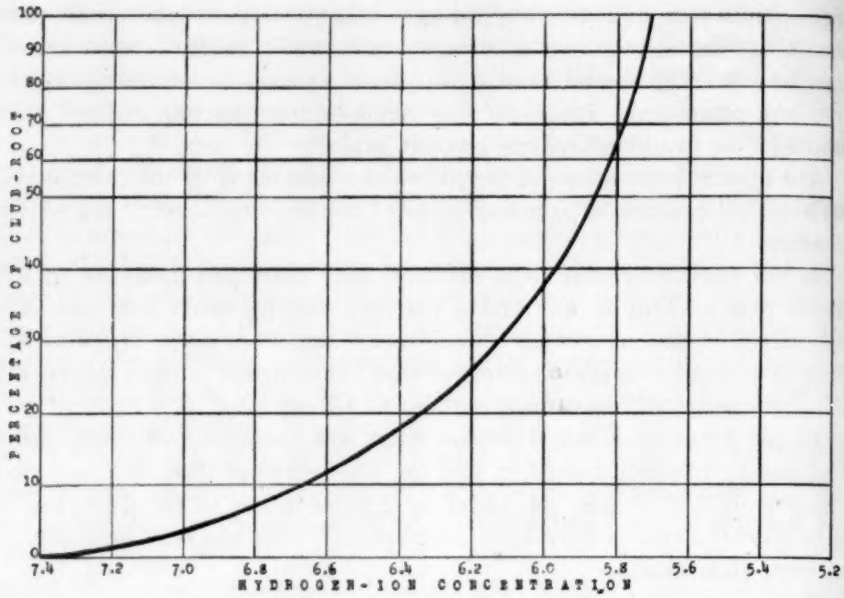

FIG. 1. The effect of soil acidity on the percentage of club-root.

is inhibited completely is not very definite. A trace of the disease was obtained on plants in greenhouse soil ranging from pH 7.2 to 7.4. Only one diseased root was ever found in soil that tested higher than this. Between pH 7.2 and 6.0 there was a rapidly increasing amount of club-root, until at a point below pH 6.0 it was possible to get almost 100 per cent of infection (figure 1). In fact, in a few cases both in the greenhouse and outdoors there was over 80 per cent of disease in soil that tested pH 6.6. These variations apparently were not due to different types of soil, for there seemed little or no difference in the use of dark loam sent from New York State as compared with the sandy clay of New Jersey.

Previous workers have raised the question as to whether the lime acts as a fungicide or merely retards the growth of the fungus. Ravn (11) did not believe it killed the spores, because quick-lime was no more effective in the production of a healthy crop of crucifers than was hydrated lime. The variation in hydrogen-ions of the soil in the jars used in the present experiment gave an opportunity for making further observations on this point. It was found that, when lime was added so that the soil tested considerably higher than neutral, no infection of the cabbage roots occurred. But when successive crops were grown in the same jar, the acidity of the soil finally

became high enough to permit infection of a large percentage of roots, even though no additional inoculum was added.

Hollrung (6) states that sulfur has been recommended for the control of club-root but adds that he does not know how effective it is. Müller-Thürgau and Osterwalder (10) attempted to control the disease by using a 1 to 3 mixture of sulfur and lime. In some cases it was broadcast, while in others it was mixed with the soil in the holes where the cabbages were to be set; in either case there was little or no control of the disease. As a result of using hydrated lime or stone lime, however, 80 to 86 per cent of the plants were clean. Collinge (2) gave as his opinion that a fall dressing of lime (1,500 lbs. an acre) followed by a spring dressing of sulfur (500 lbs. an acre) was better than lime alone.

In the field work, sulfur was compared with lime. In one plot 320 lbs. an acre of inoculated sulfur were raked into the top layer of soil before the cabbage was planted. In another plot 640 lbs. were applied.

TABLE 3.—*The effect of applications of sulfur on the development of club-root of cabbage. Field experiments at New Brunswick, New Jersey*

Soil treatment	First planting		Second planting		Third planting	
	No. plants	Percentage disease	No. plants	Percentage disease	No. plants	Percentage disease
Check	348	43.7	526	83.1	366	93.8
320 lbs. sulfur per acre	301	28.6	466	45.0	110	91.0
640 lbs. sulfur per acre	332	28.0	506	48.9	23	80.0
3000 lbs. lime per acre	261	28.8	401	42.5	425	3.2

The results of this test, presented in table 3, show that at first club-root was reduced slightly, and that the plants did not seem to be injured by the inoculated sulfur. But the third planting showed how sensitive cabbage is to sulfur injury. In addition, when sulfur was applied in the larger quantity, there seemed to be only a slight decrease in the amount of disease. Consequently this fungicide does not appear to be of value in the control of club-root.

SUMMARY

Investigators have long recognized that lime used in large quantities reduced the amount of club-root.

The present work shows that, under the conditions described above, pH 7.2 to 7.4 is the upper limit at which *Plasmodiophora brassicae* Wor. causes infection. The amount of disease increases very rapidly with an

increase in soil acidity. At pH 6.0 or even above, 100 per cent infection is possible.

Sulfur injures the cabbage plant and does not control club-root.

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VARIETAL SUSCEPTIBILITY OF POTATOES TO FUSARIUM WILT AND STEM-END ROT[1]

R. W. Goss

Observations made during the past seven years indicate that there is considerable variation in the susceptibility of the potato varieties grown in Nebraska, to Fusarium wilt and stem-end rot previously described by the author.[2] It has not been uncommon to find fields of Irish Cobblers with a much higher percentage of wilt than Bliss Triumphs planted in the same field and growing under similar conditions. A careful check of the seed used in a number of instances indicated that the infection was chiefly from the soil and that the differences in the amount of infection might possibly be explained on the basis of varietal susceptibility.

In the winter of 1925–26, a preliminary test was made in the greenhouse. Healthy potatoes of the Triumph and Cobbler varieties were planted in soil that had been sterilized and subsequently inoculated with pure cultures of *Fusarium eumartii*. While only 30 plants of each variety were used, the differences in the amount of infection were very marked. The Triumph plants showed 30 per cent slight wilt; while 90 per cent of the Cobblers were wilted, and most of the wilt was of a severe type, resulting in the death of the plants. It was found, by examination of the underground portion of the plants, that 80 per cent of the Triumphs had slightly infected roots and 100 per cent of the Cobblers were severely infected. The stems of 70 per cent of the Triumphs were healthy, while all of the stems of the Cobblers showed some discoloration. The high prevalence of root infection in the Triumphs, together with the lightness of the infection as indicated by the lack of any severe wilt, indicated that this variety did have some degree of resistance to the disease as compared with the Cobblers.

In 1926 a test was conducted in the field by planting healthy Irish Cobblers and Bliss Triumphs in alternate rows on land that had produced a crop of potatoes the previous year with a fairly high percentage of wilt. One half of the seed potatoes of each variety was treated with a 1–10 solution of Semesan Bel after cutting. This was done to determine whether an organic mercury disinfectant used in this way would prevent infection of the seed piece from the soil.

[1] Published with the approval of the Director as Paper No. 47, Journal Series, of the Nebraska Agricultural Experiment Station, Lincoln, Nebr.

[2] Goss, R. W., Potato wilt and stem-end rot caused by *Fusarium eumartii*. Neb. Agr. Exp. Sta. Res. Bul. 27. 1924.

In 1927 a similar test was conducted in which Early Ohio potatoes were included. Half of each variety was again treated with Semesan Bel, and the untreated and treated seed pieces were planted in alternate, 150-hill rows on land presumably infested with *Fusarium eumartii*.

Both in 1926 and 1927 the climatic conditions were unfavorable for the development of wilt in the field: fairly low temperatures with considerable precipitation prevented the development of severe symptoms. When the potatoes were dug and examined it was found that some infection had occurred even though no foliage symptoms had appeared. Every tuber was cut and examined for stem-end rot and vascular discoloration. The results are presented in table 1. Most of the infection occurred as a vas-

TABLE 1.—*The effect of seed treatment on percentage of wilt infection in three varieties of potato. Based upon the weight of infected tubers*

Variety	Treatment	Percentage of wilt[a]	
		1926	1927
Triumph	None	15.6	5.7
do	Semesan Bel	9.6	9.7
Cobbler	None	28.9	10.6
do	Semesan Bel	24.7	12.1
Early Ohio	None	—	13.1
do	Semesan Bel	—	13.3

[a] The percentages are based upon the total weight of potatoes from 300 hills for each treatment of Triumphs and Cobblers in 1926 and 450 in 1927. Only 300 hills of Early Ohio were used for each treatment.

cular discoloration, typically a band of soft brown tissue from one-eighth to one-fourth of an inch wide, extending in a few cases through the entire tuber. Quite often this vascular discoloration was associated with a stem-end rot, which was characterized by sunken areas and from which *Fusarium eumartii* was isolated.

The results presented in table 1 show that the Irish Cobbler and Early Ohio varieties are equally susceptible and that they are both much more susceptible than Bliss Triumph. In the untreated rows of each variety only about half as much infection occurred in the Triumphs as in the other two varieties. The difference between these varieties appears to be chiefly in degree of susceptibility. The writer does not intend to convey the impression that the Triumph does not become severely infected with wilt. Many fields of Triumphs have been observed with from 30 to 50 per cent of wilt infection, and the disease in this variety is one of the worst with which the grower has to contend in western Nebraska. The greater sus-

ceptibility of the other varieties, however, means that they should never be planted on heavily infested soils.

The results with seed treatments are not so clear cut. In 1926 there was some decrease in infection when seed was treated with Semesan Bel, but in 1927 the treatment apparently had no beneficial effect and there was even a slight increase in the amount of disease. Either the disinfectant is of no great value in protecting the seed piece from infection or else infection takes place through the roots. Root infection is considered to be the more common method of invasion in Nebraska.

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PHYTOPATHOLOGICAL NOTES

A "Streak" of Tomatoes Produced by a Disturbing Principle from Apparently Healthy Potatoes in Combination with Tomato Mosaic Virus.[1]—An investigation of the nature of a severe mosaic disease of the tomato prevalent in the commercial tomato fields of Utah has identified the disease as "streak" of tomatoes. This disease has been shown by T. C. Vanterpool[2] to be produced by the combination of tomato mosaic and potato mosaic viruses in the infected tomato plant. During the course of the investigations evidence has been obtained to the effect that apparently healthy potatoes of certain commercial varieties carry some disturbing principle which, when in combination with tomato mosaic virus, is capable of producing the same set of symptoms as characterize streak.

Green Mountain, Early Ohio, and Irish Cobbler potatoes have been used as sources of inoculum for inoculation experiments with healthy potato juices. The Green Mountain potatoes were sent by Dr. E. S. Schultz, of the United States Department of Agriculture, to the Department of Plant Pathology of the Utah Agricultural Experiment Station for the purpose of conducting mosaic-disease investigations. The Early Ohio potatoes were grown on the Station potato seed plots and indexed in the Station greenhouse. The Irish Cobbler potatoes were sent by Dr. M. B. McKay, of the United States Department of Agriculture (located at Corvallis, Oregon), for work on the new disease of potatoes prevalent in Utah this last summer (1927).

The Green Mountain and the Early Ohio tubers producing the vines from which the juices were obtained for the inoculation experiments were indexed in the Station greenhouse in the early spring of 1926. From appearance they seemed to be free from all visible virus troubles. The Irish Cobbler tuber was cut in half and both halves planted in the greenhouse under celluloid cages. Both halves produced apparently healthy plants. So far as could be observed, all the plants from which juices were obtained were perfectly healthy and normal. Juices from these potato plants inoculated into healthy tomato plants (inoculated at the same time with tomato-mosaic virus) produced symptoms of "streak" as severe as, and—under the conditions observed—indistinguishable from, those of the "streak" produced by the combination of tomato-mosaic and potato-mosaic viruses on the tomato. Further investigations are in progress at this Station.—H. L. Blood, Department of Plant Pathology, Utah Agricultural Experiment Station, Logan, Utah.

[1] Approved for publication by the Director, December 22, 1927.

[2] Vanterpool, T. C. Streak or winter blight of tomato in Quebec. Phytopath. 16: 311–331. 1926.

Photographic collection of Erwin F. Smith. The photographic collection of the late Dr. Erwin F. Smith has, through the generosity of his colleagues, been placed in the hands of Science Service. This consists of about 200 negatives, together with a large number of prints. These are mostly of persons now active in phytopathology, though the collection also contains many portraits of notable figures in the history of botany.

The collection is now being arranged and catalogued. After they have been properly labeled, all the portraits, together with a complete set of prints from the negatives, will be deposited with the Library of the U. S. Department of Agriculture. The negatives will be retained by Science Service. A special list of these will be issued in the near future, so that phytopathologists and botanists generally may have an opportunity to secure photographs of their fellow-workers.

Correction. Correction for "Virus mixtures that may not be detected in young tobacco plants" by H. H. McKinney, Phytopath. **16**: 893.—The following mistake occurs in my paper, reference to which is given above:

line 8, *read* 32.5°–37.5° C. *for* 90°–100° C.

Inasmuch as the list of errata for volume 16 already has been published, I am taking this means of calling attention to the error.—H. H. MCKINNEY, U. S. Department of Agriculture.